Vol. 38, No. 1 March 1951

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

BY

R. W. MARSH

AND

I. THOMAS

WITH THE ASSISTANCE OF

F. J. ANSCOMBE J. T. MARTIN
F. C. BAWDEN D. J. WATSON

CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE, N.W.1
NEW YORK: 51 MADISON AVENUE

COMMONWEALTH MYCOLOGICAL INSTITUTE
APR 1952

The Association of Applied Biologists

President: R. W. MARSH, M.A.

Vice-Presidents: J. W. EVANS, M.A., D.Sc. AND H. C. GOUGH, PH.D.

Hon. Treasurer
H. F. BARNES, M.A., PH.D.
Rothamsted Experimental Station,
Harpenden, Herts.

Hon. Editor
R. W. MARSH, M.A.
Research Station,
Long Ashton, Bristol

Hon. Assistant Editor
I. THOMAS, M.Sc., PH.D.
Plant Pathology Laboratory,
Milton Road, Harpenden, Herts.

Hon. Secretary
R. K. S. WOOD, A.R.C.Sc., PH.D.
Imperial College,
London, S.W. 7

Hon. Secretary (Programmes)
L. BROADBENT, B.Sc., PH.D.
Rothamsted Experimental Station,
Harpenden, Herts.

Council

PROF. J. B. CRAGG, M.Sc.
J. W. EVANS, M.A., D.Sc.
C. E. FOISTER, PH.D.
MISS M. T. FRANKLIN, PH.D.
S. D. GARRETT, M.A., Sc.D.
H. C. GOUGH, PH.D.
R. M. GREENSLADE, PH.D.
R. V. HARRIS, D.Sc.
I. ISAAC, PH.D.
A. B. P. PAGE, D.Sc.
I. W. PRENTICE, PH.D.
MRS M. A. WATSON, PH.D.

Plant Pests and Diseases Committee

Hon. Secretary: N. G. MORGAN, B.Sc., Research Station, Long Ashton, Bristol

THE STRUCTURE AND FORMATION OF THE EGG OF THE FRUIT TREE RED SPIDER MITE, *METATETRANYCHUS ULMI* KOCH

BY J. W. L. BEAMENT, M.A., PH.D.

Agricultural Research Council, Unit of Insect Physiology, Department of Zoology, Cambridge

(With 8 Text-figures)

The summer egg, and the overwintering diapausing egg of the fruit tree red spider mite, *Metatetranychus ulmi* Koch are described in detail; there is no reliable factor by which these two may be distinguished on inspection.

Both types of egg have a common basic shell structure, consisting of an outer thick wax layer of very high melting point and a cement layer of oil and protein which attaches the wax to the underlying 'shell' layer enclosing the living material.

The shell layer is extremely resistant to penetration and to attack by chemicals or solvents; it appears to be composed of a material similar to keratin. This layer is formed in the ovary, towards the end of yolk accumulation. The egg is apparently fertilized precociously, and there are no associated nurse or follicle cells; only one egg is matured at a time.

The female reproductive system is described in some detail; the egg receives its shell layer in the simple sac-like ovary, and then passes into a glandular ovipositing pouch which is evaginated through the genital aperture at oviposition. The shell layer makes contact with the substrate, and the pouch secretes the cement over the rest of the shell layer so that the egg adheres to the substrate by a ring of cement around the base. The outer wax is then secreted over the cement, and it also leaves a large hole at the base of the egg, bordered by the cement.

The female leaves the egg by rotating into an almost vertical position. In this process the ovipositing pouch is drawn off the egg, and withdrawn into the female; consequently, the wax, which is plastic when first secreted, is drawn up into the characteristic spike which surmounts the egg. The spike is of no further physiological significance to the egg.

Owing to the hole in the egg-coverings at the base, the shell structure is not waterproof. The developing organism waterproofs the summer egg by secreting a wax layer into the inside of the shell about 6 hr. before laying, previous to which the egg will only survive in humidities greater than 85% R.H. After waterproofing, the egg readily develops in humidities of 30% R.H.

The waterproofing wax has a transition point of 68° C. The shell of the winter egg is similarly composed and waterproofed, but winter eggs are held up in the female until a later stage of embryonic development, so that they are waterproofed when they are deposited on the bark.

Some discussion is made of the importance of leaf and bark humidities in relation to the different times at which summer and winter eggs are waterproofed.

The fruit tree red spider mite *Metatetranychus ulmi* Koch (=*Paratetranychus pilosus* C. & F.) was first noted on fruit trees in the British Isles by Theobald (1911). From that time onwards it appears to have been found in small numbers, and with no commercial significance, in most parts of the country. Recently, however, this species has assumed a dominant position among the arthropods infesting fruit trees, particularly in the orchards of Essex and Kent. The present work sets out to investigate the structure and physiology of the egg of the mite, to gain knowledge of the most vulnerable stages in its life history, and possibly to correlate this work with control measures.

Relation of egg to life history of mite

From the work of Newcomer & Yothers (1929), Cagle (1946), etc., it is generally recognized that the life history of the fruit tree red spider mite is somewhat as follows. Overwintering eggs hatch between April and July, giving rise to males and females of a 'summer generation' type. This generation produces eggs which hatch in approximately 12 days to give a second summer generation. The actual number of these successive summer generations (each lasting about 28 days) may vary from perhaps one or two or even seven (under American conditions of climate; Newcomer & Yothers, 1929); the number apparently depends particularly on the prevailing climatic conditions. Nevertheless, under field conditions, females of any of these generations may produce eggs which survive the winter in a state of diapause and give rise to summer generations the following year. These 'winter' eggs may be oviposited between July and October.

There are thus two kinds of eggs to be considered: two kinds which at the outset of this work were quite indistinguishable if isolated specimens were inspected. The majority of non-diapausing eggs are laid on a leaf surface in nature; almost all the over-wintering eggs are deposited on the bark of trees, but it has been recorded that summer eggs have been laid on bark, and winter eggs on leaves, so that this factor is not, of itself, a reliable criterion. A statistical difference in size exists between the two types of egg, the winter egg being slightly larger (Blair, 1949), but this is by no means sufficient to separate eggs into two classes. On the average, winter eggs are also darker than summer eggs, but the natural variation in colour is such as to make this an impracticable means of separating or identifying them. It was hoped that a study of the egg structure might lead to some reliable method of distinguishing between the two types of egg, for this problem was a source of difficulty throughout the course of this work.

Nomenclature

Eggs which, under normal favourable circumstances would hatch within 3 weeks of oviposition are termed *summer eggs*: the parent producing them is a *summer egg-laying female*, or more simply, *summer female*; eggs showing a diapause, and requiring the elimination of this state before completing development are called *winter eggs* or *diapause eggs* and their progenitors, *winter egg-laying*, or *winter females*.

Material

Sources. Supplies of winter eggs in diapause were obtained from orchards in Essex, Kent and Somerset: of summer and winter egg-laying generations from eggs in which diapause had been eliminated by suitable temperature and light treatment (Lees, 1950 and unpublished work).

Culture methods. Stock cultures were maintained on potted apple seedlings which were replaced at regular intervals (Massee, 1947) to ensure that the mites were feeding from vigorous plants. Seedlings bearing cultures were kept in the window of the laboratory during summer months, but at other times of the year were placed in a special cabinet. Here, the natural daylight was augmented to a full summer day length by a combination of fluorescent and tungsten filament lighting. Temperature was controlled and fluctuated between a 'day' and 'night' value and air circulation and humidity conditions were controlled. With the aid of this apparatus it was possible to approach the climatic conditions of any time of the year.

The major part of the work on basic egg-shell structure was done on eggs obtained from the field—more detailed work, involving a knowledge of the age of the egg made use of eggs obtained by confining one or more females on single leaves floating on a water surface (for summer eggs). Single leaves were also used, supported on a surface of moist sand, with the female mite imprisoned in a glass capsule sealed to the leaf by gelatin. From this latter method of culture, winter eggs were occasionally obtained laid on glass, thus allowing a detailed examination of the point of attachment of the egg.

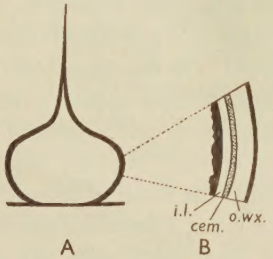


Fig. 1. A, egg of *Metatetranychus ulmi*, side view, approximately ×500. B, enlarged schematic section of shell of newly laid summer egg. *cem.*, cement; *i.l.*, inner layer; *o.wx.*, outer wax layer.

Basic structure of the egg-shell

The summer and winter egg-shells of *Metatetranychus ulmi* are very similar, and the description given here refers equally well to both types of egg. The egg is spherical in shape, slightly flattened dorsoventrally, with a spine projecting centrally from the upper surface (Fig. 1 A). The shell is transparent and all colour is due to the living contents of the envelope. The average diameter of summer eggs is 0·144 mm., of

winter eggs 0·147 mm. The spine has a length of about 0·2 mm. but is subject to extreme variation in size and length (see below p. 13). The egg is attached by its flattened base to the substrate on which it is found. The principal layers of the shell of both winter and summer eggs would appear to be identical; a section through the shell is illustrated in Fig. 1 B. There are three layers—an outer wax, a cement layer and an inner 'shell' layer. Owing to the method of formation of these structures, the term 'chorion' is avoided (see below p. 9, also Beament, 1946*a*, Lees & Beament, 1948).

The outer layer

The outer surface of a red spider mite egg is exceptionally hydrofuge; the egg is covered by a hard, transparent waxy coating, somewhat more than 1μ thick, uneven, but not irregular in thickness, and sufficiently substantial to be removed intact from the egg by a microdissection needle. It remains unchanged in shape if the egg contents shrivel within it and is fractured around the equator of the egg when hatching occurs.

Properties of the layer. The outer layer of the shell appeared to be a waxy material, and the effect of organic solvents was first tried. The material is slightly soluble in cold chloroform, readily so in boiling chloroform and hot pyridine, less so in boiling benzene. It is, however, insoluble in hot or cold lower alcohols, carbon tetrachloride, ethyl ether, petroleum ether or acetone, though prolonged treatment with some of these may produce a degree of plasticity which can be detected with a needle. This solubility suggests a hard wax of the type found on the cuticles of adult *Ornithodorus moubata* (Lees & Beament, 1948). It is extremely resistant to destructive solvents: cold alkalis have no effect, and it appears to melt in, rather than dissolve in, fused potash. Neither hydrochloric, nor cold nitric acids have any effect. These properties are typical of inert lipoid material. On the other hand some strong oxidizing agents have a marked effect. Although sodium hypochlorite solution and nitric acid are without apparent action, fuming nitric acid saturated with potassium chlorate, and heated, breaks the layer down to oily droplets, and it is also dissolved by liquid bromine. This would suggest that the material present is not a saturated compound, or that a major component of it is not a saturated paraffin. In the circumstances it is surprising that glacial formic acid, acetic acid and even saturated formaldehyde solutions have some solvent effect, if not actual chemical action: this can only be explained by assuming that they have special affinity with some group or molecular structure present.

All these experiments lead to the conclusion that the outer layer of the shell contains a large proportion of inert lipoid material, of an unsaturated nature. This hypothesis is considerably strengthened by the evidence from staining reactions, and tests for protein. The material did not take up any of a large series of aqueous stains which were used (such as neutral red, basic and acid fuchsin, safranin, rosanalin, etc.) and was completely uncoloured when sections cut with a freezing microtome were stained in haematoxylin, haematin, etc. In order to make sure that the extremely

hydrofuge nature of the material did not prevent these stains from wetting the surface, some of them were applied in solutions of 70% alcohol, which wets the outer layer readily, but without any visible effect. Most of these tests were made on fragments of the layer, removed from the shell by microdissection. An irregularly distributed coloration on the inside was often detected, and attributed to the presence of small quantities of cement which adhered. No method was found of obtaining the layer intact but free of cement and on such a small scale it was sometimes difficult to separate coloration due to cement residues, from coloration of the layer; the evidence from experiments was only taken as conclusive when it was obvious that coloration was due to a residue on the inner surface of the outer layer. The results of such experiments with ninhydrine, picric acid, *p*-benzoquinone, ammoniacal silver nitrate, and the xanthoproteic reaction, showed conclusively that no proteinaceous material was present.

On the other hand, the outer layer does not stain with any of the Sudan series of dyes, with Ciba's B.Z.L., or with osmic acid. This latter is perhaps unexpected. None of the cuticular waxes of insects or insect eggs so far examined respond to these substances (Beament, 1945, 1946*b*, etc.) but they are considered to be saturated compounds, while the evidence above suggests that this is an unsaturated material, and it should therefore reduce osmic acid.

These results were upheld when the properties of extracted material were examined. Large numbers of whole live winter eggs were washed in warm chloroform, and the resultant solution filtered, and allowed to evaporate. The residue was a very hard, white waxy substance, which appeared inert and non-reactive to any of the tests outlined above. It did, however, show one rather important difference. If a piece of this layer, obtained by dissection from the egg-shell, is heated in a melting-point tube, it melts sharply at 171° C.; the isolated material melts somewhat more diffusely at about 154° C. A reason for this apparent discrepancy is suggested below.

The evidence suggests that the outer layer of the red spider mite egg-shell is composed of a hard wax, of very high melting point. It has a thickness of about 1μ, which varies somewhat from specimen to specimen, and although its position resembles that of the outer waterproofing wax on the eggs of the ticks, *Ornithodorus moubata*, and *Ixodes ricinus*, and even, perhaps, the waterproofing wax layer on the cuticle of insects (Wigglesworth, 1945; Beament, 1945) its function is not, primarily, one of waterproofing (see p. 15).

The cement layer

Underlying the outer layer, and attaching it to the shell layer of the egg, is the *cement layer*. This name has been chosen, because in addition to its connective role in the shell, this substance affixes the egg to the substrate, and also shows considerable affinity of composition to the egg-cements of insects (Beament, 1948 and unpublished). The cement layer, as might be expected of a layer with this function, varies in thickness, but is usually about 1μ thick. It is soft and pliable, and, surprisingly,

is still capable of considerable elastic extension even after winter eggs have been exposed on trees in the open for a period of months.

Properties of cement. The cement, which is either a very viscous liquid or an extremely plastic solid, is not a homogeneous substance but made up of a large number of granules of approximately 0·2–0·5μ diam., embedded in a matrix which must comprise the adhesive portion of the material. The granules stain very deeply in many aqueous stains; such as safranin (at a pH 11·5) and basic fuchsin, though slightly less strongly in Orange G (pH 2) (Michaelis & Goddard, 1934), and acid fuchsin. These reactions immediately suggest that the granules are composed of protein, and such tests as the xanthoproteic confirmed this idea, while darkening and tanning was intense with *p*-benzoquinone solution. The ninhydrine test was, however, very doubtfully positive. No staining was ever observed with the Sudan stains, Ciba's B.Z.L., or osmic acid, and it seems that the granules contain no oil. This conclusion is upheld by the action of corrosive substances. Nitric acid, liquid bromine, potash, diaphanol, and sodium hypochlorite all break down the granules without revealing any oil droplets. It is perhaps surprising that the granules are also attacked by glacial formic acid and by concentrated sulphuric acid; the majority of the components of insect egg-shells are extremely resistant to these reagents (Slifer, 1937; Jahn, 1935; Davies, 1948; Beament, 1946*a*, 1948). These granules appear to be composed almost entirely of protein, which is not water soluble or water miscible.

The matrix, on the other hand, certainly is not composed entirely of protein. It would appear to contain the same proteinaceous material which makes up the granules, but dispersed in the plastic solid; all the staining reactions listed above occur, but to a less intense degree. In addition, it is blackened by osmic acid, and colours with the Sudan stains. When strong nitric acid is added, oily droplets are produced which can be identified by their staining reactions as oil, though no discrete droplets are discernible in the matrix under the highest magnification. When whole shells are placed in chloroform, the outer wax dissolves away, and the cement is considerably modified. A white residue is left which proves to be the granules, unmodified, and a 'precipitate' of proteinaceous material, from the matrix. This latter material has almost identical properties with the granules; the experiments lead to the conclusion that the matrix consists of the same protein as the granules but finely admixed in physical solution with oil, which is removed by the solvent action of the chloroform. The matrix is similarly broken down, though less vigorously by other oil solvents such as benzene, carbon tetrachloride, ethyl ether or petroleum ether.

It is concluded that the matrix of the cement—that part which is apparently responsible for the adhesive properties of the substance—is composed of protein and oil; the staining of the residue after extraction with chloroform is much more intense than that of the normal cement, indicating that the oil protects the protein components against invasion and staining by aqueous materials. Since no individual oil droplets can be demonstrated, and since simple solvent action will separate the

protein and oil components, the cement matrix must consist of a very intimate admixture, or even solution of protein in oil, but it cannot be termed a lipoprotein. The hyaline nature of the material suggests a physical solution. How far the lipoid portion of the matrix is essential to the adhesive properties of the cement is not obvious; washing eggs on bark with chloroform is not a very efficient method of removing them in large numbers, and does not seem to destroy the adhesive properties of the cement once it has been laid down.

The waxes obtained by washing whole eggs in chloroform must inevitably contain oil from the matrix and this probably accounts for the variation in properties of the extracted wax as compared with the natural outer layer noted above. In constitution, the cement is very similar to that which *Rhodnius* uses to cement its eggs to the substrate, but in *Rhodnius* (Beament, 1948) the initially plastic and adhesive cement rapidly becomes brittle and solidifies; the cement of the egg of *Aphis pomi* Deg. or *A. fabae* L. (Beament unpublished) on the other hand, retains its plasticity for very long periods, though this material contains large visible oily droplets.

The inner or 'shell' layer

The only envelope covering the egg of the red spider mite, which can reasonably be called a shell layer is that which completely surrounds the yolk contents when the egg is laid, and which lies below and adhering to the cement. It is a thin transparent lamina (less than $0{\cdot}5\mu$ thick) and is exceedingly tough and flexible, though not at all elastic.

Properties of the shell layer. It is difficult to obtain a great deal of positive evidence on the composition of this membrane, since it is composed of a most inert substance. It does give a very weak xanthoproteic reaction, and stains with fuchsin stains, picric acid and iodine: more strongly in Orange G than in Safranin. These are reactions which would be given by proteinaceous material. On the other hand, there is no reaction with ninhydrine, or with *p*-benzoquinone, even on heating, so that such protein materials are certainly considerably modified. Tests with solvents illustrate this further: nitric acid, even on heating, attacks the membrane very slowly; hot fuming nitric acid saturated with potassium chlorate does so only slightly more rapidly. Fused potash, diaphanol and sodium hypochlorite all act extremely slowly in dissolving it, while concentrated sulphuric acid appears to modify it without solution. Hydrochloric acid has no swelling effect at all, and while it is macerated by liquid bromine, it is quite unaffected by potassium permanganate.

In all of these reactions, there is a striking resemblance to the reactions of the epembryonic membrane of the *Rhodnius* egg. Beament (1949) suggested that the peculiar properties of this membrane might be due to cystine linkages, between the protein molecules present; similar properties of the chorion of *Rhodnius* (Beament, 1946*a*) are attributed to the presence of 2–3% sulphur which has been shown present in many egg-shells (Verson, 1884; Tichomiroff, 1885). The usual elemental tests for sulphur were applied to residues of several membranes without success, though the

actual amount of sulphur present, even if the shell layer did contain 2% of sulphur, would be exceedingly small. However, some support for the idea that this layer is vulcanized, was obtained from the following series of experiments.

If sulphur is present, then the membrane might show some resemblance to either silk, or keratin. The enzymes, trypsin and pepsin were without effect, and so also were silk solvents such as lithium iodide, calcium sulphide etc., but a solution of thioglycolic acid, at pH 12, as recommended by Michaelis & Goddard (1934) was applied to the membrane and had a considerable softening and plasticizing effect. After the action of this reagent, the residue dissolved rapidly in nitric acid, potash, hypochlorite, etc. This certainly seems to indicate that the main resistance of the shell layer is due to linkages of the –S–S– type, and, with some hesitation it may be implied that there is some chemical affinity between this substance and keratin.

Apart from the example of the *Rhodnius* egg-membrane quoted above, layers of similar properties have been found in other eggs. The inner membrane of tick eggs (Lees & Beament, 1948) and layers in eggs of *Psylla mali*, etc. (Beament unpublished) all fall into the same category of chemical substance, and must all be allied to keratin. However, the intensity of a staining reaction on a thin lamina is not a reliable indication of the activity of the material under consideration. Aqueous stains, for example, are more intense in their coloration of the shell membrane after it has been extracted in chloroform, than when removed mechanically from the egg. But the cut edge of a membrane will stain just as intensely as the extracted membrane does in its entirety, and it can be shown (see below) that the shell layer is protected from the invasion of aqueous materials by lipoid material on either side of it. The membrane does not stain at all with fat stains, and there is no evidence for supposing that this membrane contains any lipoid scattered throughout its thickness, or that there is a differential penetration factor (Hurst, 1948).

Conclusions. The descriptions given above indicate the properties of the three layers comprising the protective coverings of the red spider mite egg: outer wax, cement and shell layer. But the egg is not covered uniformly by these materials; for example, there is the surmounting spike described above, and the apparent anomaly that the egg is attached to the substrate by the cement which is not the outside layer of the greater portion of the shell. However, to understand the final construction of the egg-shell, it is essential to study the structure of the female reproductive system, and the mode of oviposition in some detail, since the final form of the egg coverings is a result of the method of egg-laying. The summer egg-laying female is considered first.

Reproductive system

Summer female

The ovary. The ovary of the summer female of *Metatetranychus ulmi* is contained in a large sac; in the mature specimen it occupies the greater portion of the ventral abdominal cavity (Fig. 2). It contains numerous cells with large nuclei staining

heavily in haematoxylin, haemalum, etc., with comparatively small cytoplasmic content and irregularly shaped owing to the tight packing of the ovarian contents. The wall of the ovary appears to be a thin, non-cellular membrane and the contents all seem to be immature oocytes. All except one of these cells are very much smaller than the size of the egg laid at oviposition. From sections and from examination of whole ovaries, one cannot easily decide whether any of these cells play a nutritional role, as 'nurse' cells, but the staining properties throughout oögenesis do not indicate any differentiation of function. The exceptional cell is the next egg to be deposited, though its size depends on the time which has elapsed since the previous egg was laid. Immediately before the oviposition mechanism starts it occupies a greater volume of the ovary than the whole of the rest of the contents. Only one egg, therefore, is matured at a time, presumably simply due to the final very large size of the egg compared with the size of the female.



Fig. 2. Median longitudinal section of mature female mite, showing relationship of reproductive apparatus. *a.g.c.*, anterior gut cavity; *an.*, anus; *ap.pch.*, aperture from ovary to pouch; *c.n.m.*, central nervous mass; *ft.*, fat deposit; *mat. egg*, mature egg; *m.*, mouth; *m.can.*, muscular section of genital canal; *ov.*, ovary; *ov.pch.*, ovipositing pouch; *p.g.c.*, posterior gut and excretory cavity; *rep.ap.*, reproductive opening.

Until a comparatively late stage in the process of yolk accumulation, the egg appears to have no other outer covering than the protoplasmic surface of the egg-cell. In this respect it is similar to insect eggs rather than those of ticks which form shells comparatively early in development and accumulate most of the yolk *through* an incomplete shell layer (Lees & Beament, 1948).

The shell appears to be laid down in the ovary itself, and as the final process of activity in the ovary; in view of the absence of any associated nurse, follicle, or ovarian wall cells, it is undoubtedly a product of the egg-cell itself. For this reason, the shell layer cannot be called a 'chorion' (Beament, 1946*a*). Examination of eggs found very occasionally in the genital canal shows that the shell layer is apparently unmodified after its secretion, and is extremely resistant and impermeable to all but water. The contents are already under considerable hydrostatic constraint from this membrane. If the egg is not a parthenogenetic specimen, it will presumably be fertilized before the shell is added (see p. 23). It is immediately after the formation of the shell membrane that the egg passes from the ovary into the genital canal through a dorsal and posterior aperture.

The genital canal. The internal genitalia of a red spider mite female consists of a large somewhat pearshaped pouch, having a wall composed of a unicellular layer of cells (Fig. 2) which are large and columnar nearest the ovary: small with large nuclei towards the genital opening. The two regions, with totally different cell types are sharply delineated. While an egg is accumulating yolk, this organ, which will be called the *ovipositing pouch* is collapsed, the cells elongating so that it forms a compact organ with a corrugated inner surface. It is the upper, large-celled portion of this pouch which receives the egg, contained in its shell layer, from the ovary before oviposition. Sections of females (Fig. 3) chosen from cultures at suitable times show that the reception of the egg into the pouch is correlated with the activity of searching for an oviposition site, rather than with feeding. Females which had been fixed at various times after a previous egg had been laid were also compared with these preparations. This series of material indicates that under normal conditions a summer egg remains in the ovipositing pouch for comparatively little time compared with its period of yolk accumulation in the ovary. It is, however, known that the winter female is capable of retaining an egg for a long period before ovipositing it, if it is not presented with suitable environmental conditions for egg laying (and see p. 19 below).

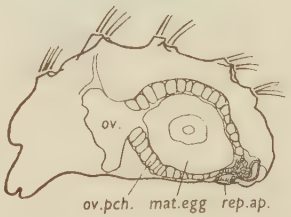


Fig. 3. Median longitudinal section of mature female mite, showing an egg in the ovipositing pouch. Abbreviations as in Fig. 2.

We can deduce that if eggs are 'held up', they are so retained in the pouch and the stimulus for a further egg to accumulate yolk depends on the delivery of an egg from the body, rather than the passage of a completed egg from the ovary.

The histology of the large columnar cells of the ovipositing pouch is peculiar. Considering the size of the mite, the cells are very large, and uniform in type throughout that part of the organ which is in contact with the egg. The fixed cells appear to contain many large granules of chromatin, scattered throughout the cytoplasm, but tending to show greater concentrations at the outer and inner surfaces and along the intercellular boundaries. The cell walls come into direct contact with the shell layer of a contained egg. The matrix of the cells stain rather erratically, but will often show a pronounced diffuse staining with fat stains, particularly with black Sudan B from solutions in 70% alcohol, applied to fresh material. No discrete wax or oil inclusions could be made out; some pouches, dissected from the mite by micromanipulation, were placed in 50% nitric acid, and gave rise to copious amounts of droplets which

could be identified as oil, but the yield varied considerably from animal to animal. In the most spectacular examples, the yield of oil was obviously very much more than might be expected from an 'unspecialized' cell. Initial staining of sections did not indicate the presence of any body which might be identified as a nucleus in these cells. A great many sections were cut, of mites of all ages, and it would appear that the nuclei of these cells break up very early in the life of the adult female stage, and that there is no normal nucleus as such in these cells while they are secreting. It was at first thought that the nuclei were very fragile, but the distribution of nuclear material in fixed cells showed that this is most unlikely. Sections fixed in Champy's fluid, and stained by Feulgen's method for nucleo-proteins, showed that the chromatin material distributed throughout the cells is of the nuclear type, whereas specimens fixed and stained immediately after moulting from the last nymphal stage appeared to have quite normal nuclei.

In all respects these cells can be classed as secretory cells, and this conclusion was certainly upheld when considering the process of oviposition, which is outlined below.

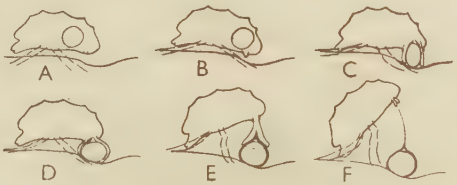


Fig. 4. Orientation of the female mite during oviposition on a leaf. A, B, taking up position; C,D, extrusion of egg and secretion of outer layers; E,F, leaving the egg and withdrawing the ovipositing pouch.

The part of the pouch nearest the genital opening, which has thinner walls, appears by comparison to be made up of non-secretory cells with normal small nuclei. There is a thin cuticular lining running some way along the inner (morphologically outer) surface, and continuous with the highly folded cuticle of the genital aperture and thus the general cuticle. Some form of minute accessory organ—a sac with very thin cellular walls, is attached to the pouch very close to the genital opening. By comparison with Blauvelt's (1945) picture of the anatomy of a female greenhouse red spider mite (*Tetranychus telarius*) it is possible that this is a bursa copulatrix, but it was not observed to contain any sperm. No other accessory glands or organs were found; the pouch is connected to the body wall of the female by a complex system of numerous long fine muscle strands.

Oviposition. Oviposition is preceded by a period of wandering, whence the female finds a suitable site to deposit the egg. When this occurs, she takes up a position (Fig. 4A) with the anterior two pairs of legs brought forward, and the hind pairs of legs placed laterally so that the ventral surface of the abdomen is slightly raised from the substrate. This does not necessarily mean that the abdomen is held in a much

elevated position, compared with the normal, for the oviposition site chosen for the summer egg is usually a slight depression in the leaf surface (see below, p. 20). There follows a short period of intense respiratory activity, shown by the rapid extension and retraction of the perispiracular plate. If the female is then lifted quickly and inverted, a white sac is seen, extruded from the genital aperture (Fig. 4 B, C, D). Examination shows that the lining of the genital aperture has been prolapsed, turning the thin-walled portion of the oviposition pouch *containing the egg in its shell layer*, into contact with the surface of the oviposition site. It is possible that the extrusion of the pouch may be effected to some extent by muscles attached to the organ itself, but pressure applied to the surface of a mature female with a needle will cause the organ to be extruded, and the general appearance of the female at this stage of the oviposition cycle suggests that the principal force causing the extrusion of the pouch is one of internal pressure from the contraction of the general musculature. Females dropped into Carnoy's fixative sometimes extruded the pouch (whether containing an egg or not) but this was completely prevented by puncturing the abdomen with a needle before fixation, when a small portion of the abdominal contents were pushed through the puncture hole.

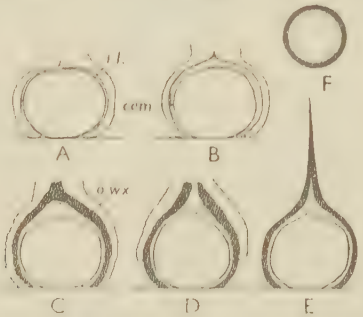


Fig. 5. Schematic sections of the egg shell during construction. A, egg in inner layer (*i.l.*), extruded against substrate in the ovipositing pouch. B, cement covering secreted (*cem.*). C, outer wax (*o.wx.*) added. D, stage in removal of the pouch showing the drawing up of wax and cement. E, construction of the spike in the complete egg. F, ring of cement enclosed by wax ring on substrate after removal of an egg.

Fig. 5 A shows the egg at this stage. A relatively large area of the shell layer is applied to the substrate, and consequently the egg is usually somewhat flattened dorso-ventrally from its natural spherical shape. If the female is lifted from the surface at this time, there appears to be no adhesion to the substrate, the egg remaining completely enclosed in the extruded pouch.

At this stage of oviposition, the egg in the pouch becomes surrounded by the cement (Fig. 5 B). This cement can only have come from the columnar cells of the pouch, since they are the only ones in contact with the egg. As the embryonic material is enclosed in the impermeable shell layer, it could not possibly have secreted

the cement, especially as this secretion is not uniform over the egg's surface. This cement covers the enclosed portion of the shell layer, and forms a ring (Fig. 5F) around the periphery of the area in contact with the substrate, thus anchoring the egg securely. Following the secretion of cement, the outer wax appears. Again, this only covers the portion of the egg which is enclosed in the ovipositing pouch, and it covers the cement, so that a further ring – this time of wax (Fig. 5C, F) – surrounds the cement ring also in contact with the substrate. The egg is thus partially surrounded by the two added layers within the extruded ovipositing pouch. The wax is secreted in a very plastic state compared with its final hard and brittle nature; this is obvious from the final stages of oviposition which take place. The absence of any of the secreted material on the substrate itself, other than the two rings mentioned above, was confirmed by a careful examination of the point of attachment of eggs laid on clean glass. On removing such eggs, two rings could be demonstrated, of cement surrounded by wax, but the central portion where the shell was applied, was still clean.

Some explanation of the highly specialized nature of the large cells in the ovipositing pouch is now possible. These cells obviously have to be capable of secreting a very large volume of, first cement and then outer wax material in a period of a few minutes. The variation in properties of individual organs is related to the particular stage which they have reached in the production and accumulation of the materials for this outpouring. The amount of oil, a substance which is a component of the cement as well as a probable precursor of the wax, is obviously related to the cyclical nature of these activities. The large amount of granular chromatin type material in the cytoplasm of the pouch cells could be associated with the intense production of large quantities of protein. The unusual nuclear distribution might be compared with that in such cells as malpighian tubes which secrete large quantities of silk in a short time (McDonnough, 1909; Bradfield, 1948). The lower cells of the pouch, more normal in histological constitution, are, apparently, non-secretory, and have as their main function, extension round the outside of the glandular portion when evagination takes place. Complex nuclear activities have also been noted in follicle cells of the ovaries of *Rhodnius* during the secretion of the chorion (Beament, 1946*b*).

The process whereby addition of wax to the egg is a function of the female recalls a similar process in ticks (Lees & Beament, 1948), but in ticks the wax which is applied to the egg by Gene's organ is the primary waterproofing layer of the egg.

When the protective coverings of the egg are complete, the female has to disconnect herself from the egg, and this she accomplishes in a rather peculiar way. Fig. 6 shows a *camera lucida* drawing of part of a whole mount obtained by fixing a female just as she was about to leave an egg; the extruding pouch is seen with the egg-shell projecting into it. In normal circumstances, the female rotates the body about the mid-lateral axis, bringing the hind pairs of legs under the abdomen (Fig. 4E, F) and extending them until she is 'standing on her head', in an almost vertical position. During this process, the ovipositing pouch (Fig. 5D, E) is drawn

upwards and off the egg, and retracted into the female's abdomen. However, the cement is plastic, and the wax is soft at this time so that they are moulded by the process of drawing the pouch off the egg. The upper hemisphere of the egg is brought to more conical proportions, and the wax is drawn up into the long spike which usually surmounts the apex of the egg. The length of this spike is usually rather less than the height of the genital aperture of the 'up-ended' female. The mode of formation of the spike obviously accounts for the variation in its length recorded above: the fact that it may be bent or almost non-existent. The spine (Fig. 5 F) is not entirely composed of wax, though this forms the upper portion of it; the lower part has a core of cement which is drawn up inside the wax, and which can be identified, though usually in a collapsed condition, when the wax has been dissolved off by treating an egg with chloroform.

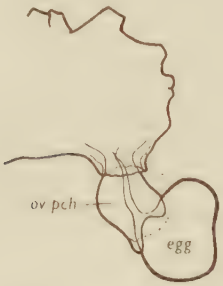


Fig. 6. Camera lucida drawing of side view; female fixed during oviposition, showing egg and partially withdrawn pouch (*ov. pch.*).

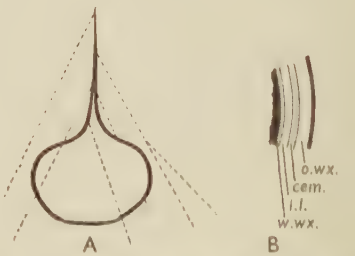


Fig. 7. A, outline of egg showing typical arrangement of guy-roping. B, diagrammatic section through waterproofed egg, showing position of waterproofing wax layer (*w.wx.*) (compare Fig. 1 B).

It would appear, therefore, that the spine has no essential function to carry out in the life of the egg, but is simply a mechanical result of the method of oviposition employed by the mite. It cannot be compared for example, with the bizarre shell structures of the eggs of many Hemiptera (Korschelt, 1887, etc.), which are apparently formed in the ovary. The spike is not infrequently broken in nature without apparent harmful effects to the egg, but if it is broken at its base, it will leave a hole in the wax layer surrounding the exposed portion of the shell—a hole filled by the cement core.

Final stage of oviposition. When the female has resumed her normal horizontal position, she turns to the egg, applying the base of the mandibles to it, and attaching a fine silk thread, which is secreted through the aperture of the spinerette. She turns from the egg to the surrounding leaf surface and attaches the other end of the silk to it, returning to the egg and repeating the process (Fig. 7 A). In this way up to a dozen 'guy ropes' of silk may be attached to an egg, usually fixed to the wax at various points along the spine and less frequently to the surface of the shell proper. The

strength and elasticity of the silk makes it extremely difficult to pick up a newly laid egg from the leaf with a needle. In the field, the silk seems always to disappear in a very short period of time, possibly due to wind action. The silk might possibly have some deterrent effect on predators while the wax is hardening, for it can readily be deformed with a needle when the egg is first laid, but becomes hard shortly afterwards. A further possible explanation of the use of 'guy ropes' is given below (p. 20).

Waterproofing in the summer egg

The above evidence shows that when the female leaves the summer egg, the latter is enclosed in three layers; only the outer of these is capable of conferring a degree of water-impermeability on the egg, and this has a large hole in it at the base of the egg where it is applied to the substrate. However, the egg is in a layer of air immediately adjacent to the transpiring leaf surface. Ramsay, Butler & Sang (1938) have shown that within a few millimetres of a transpiring leaf surface, the humidity may be markedly above the surrounding atmosphere, and that the surface of the leaf is virtually at saturation. Their experiments were made on leaves of tulip and dock; as no reference to similar measurement on apple leaves could be found, some rough experiments were made using the method of Nielsen & Thamdrup (1939), where glass tubes containing various concentrations of sulphuric acid are placed over the surface. It is reasonable to suppose from these tests that the egg is in a layer of air which is between 95 and 100% saturated.

If, immediately after laying, a summer egg is very carefully lifted from the leaf surface, taking great care not to damage the outer wax in any way, and is placed in a desiccator in dry air, it dries up very rapidly, the yolk shrivelling to a hard mass occupying about a third of the total space enclosed by the outer wax. The way in which this shrinkage takes place—first at the hole in the outer coverings, and eventually leaving the dry yolk in the upper portion of the capsule—makes it quite obvious that the principal site of waterloss is through this hole.

In high humidities such eggs will develop normally; all eggs placed in 93–100% R.H. at 18° C. developed and hatched. Below about 93% R.H., ensuing development depended on the position in which the egg was placed on a glass slide in the atmosphere under consideration. If an egg was placed flat on its base, so that the hole in the external wax was effectively covered, it would develop in humidities as low as 75%, whereas if placed on its side, or supported on a glass needle, so that the hole was freely exposed to the atmosphere, the egg dried out quite rapidly and failed to develop in atmospheres of 85% R.H. at 18° C. The outer wax cannot therefore be called a waterproofing layer, and is not similar to insect cuticle and egg-shell wax layers. It is incomplete, and the fact that the egg survives at all on the leaf surface is probably due to the high humidity in the ecoclimate of the leaf surface, combined with the close seal between leaf and egg, whence the leaf surface (possibly with its waxy coating) becomes the final layer 'protecting' the yolk.

Even so, an egg as small as that of the red spider mite—which can afford to lose extremely little water and has a very large surface area compared with its contained supply of water—could not survive for the eleven or more days necessary for complete embryonic development under these circumstances. It is not surprising that in common with other Arthropod eggs (Beament, 1946*b*; Davies, 1948) further waterproofing is carried out.

The inner wax layer. If eggs are left on the leaf, or at 95% R.H. for several hours and then transferred to atmospheres of low humidity, they will complete their embryonic development successfully in humidities as low as 30% R.H. at 18° C. regardless of whether they are placed on the base or not, and their position on the substrate seems to have no effect on the humidity in which they will survive. Experiments with a large number of eggs indicate that at about 6 hr. after oviposition, the egg becomes suddenly much more waterproof. The suddenness with which this waterproofing takes place suggests at once that a wax layer is produced by the developing organism; presumably it is secreted on to the inside of the shell layer.

Efforts to confirm the presence of such a wax layer met with two considerable obstacles. It was extremely difficult to find a suitable and reliable staining reaction, to use as an indicator of penetration, either from the yolk through a wax layer to the shell layer, or from the outside, through the shell layer to the yolk; it was also impossible at first to wash the yolk from such a minute egg and keep the shell in an uncollapsed state for the injection of stain. Without these two processes one could not carry out the usual techniques (Beament, 1946*a* and *b*, etc.) for the identification of the position of wax layers. However, these difficulties were to some extent overcome. A whole egg was embedded in a very low melting-point paraffin wax (m.p. approximately 40° C.) arranged in a thin layer on a glass coverslip. The wax was then scraped away from one side of the egg, or from the top, until a very small cap of the egg surface was exposed. The block was then immersed in distilled water, and the exposed cap punctured with a tungsten needle of a micromanipulator. The yolk contents were rapidly washed out with a micropipette, using distilled water, and the preparation could then be dried by taking up the excess fluid on filter-paper, without causing the collapse of the shell layer. Using the solutions of Safranin and Orange G referred to above, and injecting them into the shell cavity of these preparations, it was possible to demonstrate that the shell layer was stained by such liquids using preparations of eggs up to 6 hr. old, but *not* when using eggs more than 6 hr. after oviposition. The shell invariably collapsed when chloroform was injected into the cavity in an effort to remove an inner wax layer and demonstrate staining from the inside of older eggs. This was due to the effect of chloroform creeping to the outside of the preparation, dissolving the outer wax which gives rigidity to the preparation, and freeing it from the supporting embedding material. It was equally impossible to demonstrate any abrasion of a wax layer on the inside of the shell, owing to the difficulty of staining a totally wax-free surface, let alone small perforations on such a surface.

However, by taking a fragment of a washed shell, and adding water from a wax-lined micropipette, it was possible to demonstrate that the inner surface of a shell layer was readily wetted by distilled water when the shell came from an egg less than 6 hr. old, whereas when water droplets were placed on the inside of a fragment of shell from an older egg, the inner surface was obviously extremely hydrofuge. Such a fragment could be washed with chloroform, and after this, its surface properties returned to those of the pre-6-hr. type of shell.

Now Beament (1949) has shown that the developing *Rhodnius* embryo deposits a waxy material at the inside of the shell membranes during its development; and in the *Rhodnius* egg there are two waterproofing systems, one produced by the oocyte which is similar to the waterproofing wax layers of insect cuticle (Beament, 1946*b*; see Wigglesworth, 1945; Beament, 1945) as a discrete lamina, and one produced about 6 days after oviposition which probably takes the form of an impregnation of the innermost layers of the shell system. Both types of wax system lead to impermeability and to different degrees of waterproofing. The evidence outlined above, while showing that the waterproofing of the shell layer does not affect its staining properties *throughout* the thickness of the lamina (for a shell layer can be stained in a waterproofed egg from the *outside* as well as from a cut edge) does not rule out the possibility that some inner portion of the shell layer is impregnated with the wax which the developing egg secretes.

However, it is known that wax layers show sharp 'critical temperatures' at which the water impermeability of such organized structures is suddenly lost (Beament, 1945; compare Beament, 1949). When the changes in a wax-impregnation system are investigated, a breakdown in waterproofing does occur on raising the temperature, but the actual increase in permeability so effected takes place gradually.

Critical temperature of the summer egg. The usual method for the determination of the critical temperature of an insect waterproofing wax (Wigglesworth, 1945) is that of measuring, by loss of weight, the evaporation of water through the cuticle of an insect, when exposed to air at 0% R.H. at various constant temperatures for a known period of time. A typical graph constructed to show changes in evaporation rates against temperature will show a sharp break at the critical temperature or 'transition point' of the wax. Using a sensitive microbalance, this method could be applied to very small insects, and has been used (Beament, 1946*b*) for single insect eggs, such as those of *Rhodnius*. It is, however, useless if the amount of evaporation is comparable with the possible condensation on the surface of the egg between desiccation and transference to a weighing machine. It is also more satisfactory if experiments can be carried out on single eggs, rather than on uniform groups of material; the most accurate results are obviously obtained when one individual is used for recordings of evaporation rates at several successive temperatures. It would be an operation of extreme technical difficulty to determine successive temperature/waterloss values for a single red spider egg. However, Beament (1949) has shown that the transition points of a *Rhodnius* egg can be determined accurately (by comparison

with figures obtained by the original method) by the careful observation of single eggs submerged in saturated saline, instead of desiccating them in a dry atmosphere. Providing different saline solutions are used at each temperature and standardized to have approximately the same osmotic pressure, they had a water-removing effect on an egg similar to desiccation. Such experiments were made on red spider mite eggs, giving a value which could not be determined very precisely because of the optical distortion of the saline and its effect on observing a small egg with a high-powered lens system.

It is obvious, however, that in an egg as small as that of the mite, the loss of water is going to be exceedingly rapid, once waterproofing breaks down, as the surface area (even if one only takes account of the hole in the base of the egg) is extremely large compared with the total volume of water it contains. Eggs were, therefore, placed singly in typical 'melting point' tubes (fine glass capillaries with one end sealed) held by surface tension to a thermometer placed in a bath of 98% sulphuric acid, which acted as heater bath and desiccator. The neck of the bath was occluded with an air trap to prevent moist air from entering but to allow expansion; the specimen was examined through a high power binocular while the temperature was slowly raised. At a temperature of 68° C., the yolk contents suddenly dried up, leaving the outer wax *intact*, thus showing that the de-waterproofing of the egg which occurs at this temperature has no relation to the physical state of the outer wax layer, but to some other system in the shell layers. A number of determinations of the temperature for de-waterproofing, and therefore of the transition point all gave values within $\pm 0{\cdot}25°$ C. of this figure, which agrees with the value obtained, using saturated salt. The material used in these experiments were eggs of 8–10 hr. old, thus ensuring that the results were not influenced by any later changes in the shell. The observation was repeated using an oil bath, and with an exposed capsule of phosphorus pentoxide in the atmosphere over the bath, thus ensuring that sulphuric acid fumes did not play a part in the sudden collapse of the egg. (It has been shown that this acid has a very rapid effect on the eggs.) The results so obtained showed a slightly greater variation ($\pm 0{\cdot}75°$ C.) but the average figure obtained was the same.

The results of these experiments leave no doubt that waterprofing is carried out by a wax layer, having a very sharp transition point at 68° C., and without the gradual change to be expected from an impregnation system. The experiments were continued, heating the residue up to a temperature of 171° C., when the outer wax layer suddenly melted sharply. This confirmed its irrelation with the waterproofing process.

Penetration tests. The separation of a wax/protein lamina system from one in which wax impregnates a protein, depends on the following principle: if an aqueous solution penetrates a protein membrane, its transport is apparently equally opposed by a wax layer *distal* to the initial position of the penetrant, and by a wax which *impregnates* all or part of the protein. But if the protein layer also opposes, to some extent, the entry of a lipophilic—particularly a wax solvent—liquid, the passage of

that liquid will not be facilitated or affected by a wax *layer* on either side of the protein membrane, since the wax will be dissolved as soon as the solvent meets it. On the other hand (Beament, 1949) an *impregnated* membrane will certainly facilitate the entry of a lipophilic liquid, since such a liquid passes more rapidly through the interstitial lipoid rather than through 'hydrophilic' interspaces.

Washing red spider mite eggs with chloroform within 6 hr. of hatching, followed by desiccation, produces their collapse, and a collapse which takes place more rapidly than the collapse of similar non-waterproofed eggs left at 0% R.H. This again shows the water-protective value of the outer wax, though it is not 'waterproofing'. But chloroform does not penetrate at all rapidly to the yolk of these eggs, where its effect would be very noticeable (by comparison with the effect of chloroform on the yolk of such eggs punctured under the liquid.) Eggs older than 6 hr. after laying were also placed in chloroform, without any change in the observed rate of entry of chloroform. (Eggs less than 6 hr. old, placed in chloroform and left for a long time do in fact shrink slowly, due presumably to the passage of water outwards. This effect is difficult to understand, since chloroform can hardly be described as a desiccating agent. They do not do so when placed in a refined paraffin.) After 6 hr. incubation, there is no shrinkage on continuous immersion in chloroform, thus confirming the idea that an inner wax layer remains intact, and does not allow water to penetrate outwards; the chloroform has not been able to pass through the shell layer any faster after waterproofing than before.

Older eggs, placed in chloroform for several minutes and then desiccated, show no detectable increase in the rate of waterloss over similar untreated older-type eggs, in which the outer wax layer has been carefully dissected from the shell layer. The activity of the embryo in waterproofing the shell apparently makes no difference to the composition or construction of the shell layer. The egg must be waterproofed at about 6 hr. after it is laid, by a uniform wax lamina on the inner surface with a transition point of 68° C. The process is very similar to the waterproofing of the *Rhodnius* egg by the activity of the mature oocyte (Beament, 1946*b*). The structure of the egg-shell of the summer egg after 6 hr. incubation is shown in Fig. 7B.

The waterproofing of the winter egg

The outer coverings of both winter and summer eggs at the point of oviposition: outer wax, cement and shell are apparently identical; for example the melting point of the outer wax of summer eggs is 171° C., of winter eggs is 172·5° C. The mode of oviposition (p. 11) is similar, and there does not appear to be any fundamental difference in the anatomy or gross histology of the reproductive apparatus in summer egg-laying and winter egg-laying females. However, it is known that the majority of summer eggs are laid on leaves and the majority of winter eggs are deposited on the bark of the tree. It was a consideration of the difference between these two environments that led to the discovery of an essential difference between winter- and summer-type eggs.

Up to 6 hr. after laying, the summer egg is very vulnerable to desiccation, and will not survive this period in humidities lower than 75% R.H., even under special circumstances. After this time it can survive in atmospheres of 30% R.H. or even lower. But winter eggs, transferred immediately after oviposition, in many cases with the outer wax completely plastic, to an atmosphere of even 20% R.H., show absolutely no sign of desiccation, whether placed on their sides or upright; the embryos complete the first stages of development. A repetition of the experiments outlined above for summer eggs shows conclusively that the winter egg at the time of oviposition has already received its inner wax layer which has a transition point of 68° C., and is, therefore, probably the same as that of the summer egg. This difference is obviously an adaptation to the site of oviposition. Summer eggs, laid on leaves, were taken immediately on oviposition and transferred to a 'typical' bark oviposition site, where they were placed firmly on the bases. Taking account only of eggs which adhered reasonably to the bark, only two out of thirty-three eggs so transferred survived the first 6 hr., became waterproof, and completed their development successfully. It is apparent that the humidity of the bark, the normal oviposition site for winter eggs, is so low that eggs would have a very poor chance of survival if they were laid there in an unwaterproofed state and must, for efficiency, be waterproofed before oviposition.

The 'guy-roping' of summer eggs with silk fibres is possibly an adaptation to keep the hole in the base of the egg closely opposed to the substrate until waterproofing has taken place. This idea receives support from the observation that the winter egg does *not* commonly receive any guy-roping, for by this hypothesis it would have no need of them.

As a result of this discovery of a difference in waterproofing of the summer and winter eggs at oviposition, it is of interest to compare the sites of oviposition of the eggs in relation to humidity requirements.

Summer eggs. Because of their small size, summer eggs are contained within the high humidity of the layers of air saturated by transpiration from the leaf surface. However, the female mite appears to enhance the value of this naturally protecting layer by exercising some choice in the selection of an oviposition site.

Random samples of eggs laid by numbers of females on floating leaves, and on leaves taken from the field, etc., show quite strikingly, the tendency for the female to lay eggs in depressions on the leaf surface. Fig. 8 shows the typical distribution of eggs on a single heavily infested leaf taken at random in the field. 64% of the eggs are laid in the leaf veins, the majority of them in the depressions of the principal veins; of the remaining 36%, 20% are laid in other forms of obvious depression, only 16% being oviposited without apparent relation to some form of recessing of the leaf surface. This sample is typical of many which were analysed.

It may be argued that the summer egg-laying female is seeking a depression, rather than ecoclimate with high humidity; the difference in humidity between the leaf surface, and that in the sort of depression in which eggs are commonly found,

must be very small, and would be very difficult to detect, though the mites are obviously very sensitive to humidity changes. It seems possible that a slight depression would help the female mite to accommodate the extended ovipositing pouch better, and to elevate and rotate away from the egg at the end of egg-laying more easily, but this is a problem which can only be settled by a detailed study of the habits and behaviour of the egg-laying female. Suffice it to add at this stage, one further observation which must be of considerable bearing in the understanding of this problem.



Fig. 8. A random example of apple leaf from a heavily infested tree. Each dot represents an egg: those ringed by a broken line represent eggs laid in obvious hollows other than veins. An analysis of the distribution of these eggs is made in the text.

If a leaf is floated on water as a means of supporting a culture of summer females, the waxy nature of the leaf surface causes a concave water meniscus at the edge of the leaf (otherwise it is doubtful if the leaf would in fact float). This water surface does not enter into the normal possible ovipositing surface available to the mite, and summer eggs are very rarely laid round the edge of such a leaf. In normal circumstances a mite will show an avoiding reaction if it moves very close to a water surface; if it touches the water it is held at once and cannot escape under its own power; it rarely survives, even after being rescued from a water-droplet and carefully dried. Yet, if a small hole is cut, with a cork-borer, in any portion of a suitable flat leaf, and the leaf is then floated on water, the liquid rises in the hole, as the cut edges are hydrophilic, and a convex meniscus ensues which can be considered part of the available oviposition area. If females are placed on such a leaf, and the distribution of eggs is examined after a few days, it is found that a proportion, which may be as high as 31 % of the total eggs laid, are placed around the edge of the hole. The eggs are actually deposited on the leaf surface and no mite was ever found trapped in the water meniscus which protruded through the hole.

Since the 'depression' between the leaf surface and the convex meniscus cannot conceivably give the female any mechanical advantage over a plain surface as an oviposition site, this strengthens the view that the mite is seeking a place of high

humidity for oviposition of the summer egg, and that she is capable of a high degree of discrimination in such a choice. This of course raises the general problem, if mites are so sensitive to humidity, as to whether the humidity of the leaf surface changes during the bronzing effect produced by the feeding of a high population of mites, and if this is one of the factors influencing the periodic migrations which Steer (1940) and Austin & Massee (1947) have reported.

Winter eggs. Once again, there is a general tendency to lay eggs in crevices, pits, axils, etc. on the bark, but this is probably of no significance as far as humidity is concerned when the surface is not transpiring. It obviously has protective value. However, the laying of winter eggs does involve another phenomenon which reflects strikingly on the method of oviposition and shell formation.

Winter egg-laying mites, on trees where the density of the population is very high, often tend to lay many eggs in one limited area so that they fall one on top of another, and are in layers. It is obvious that many of such eggs are not going to have a smooth substrate on which the egg is mounted, forming the final surface which seals the hole in the base of the egg. Some are going to have this hole filled by the outer wax layer surface of other eggs. I am grateful to Mr M. D. Austin for drawing my attention to this phenomenon; it has since proved that some of these eggs have, upon close examination, only a minute hole in the base, which is sealed by a wax surface from another egg; in other cases, the outer wax layer extends right round the egg. It confirms the general idea that the degree of extension of the ovipositing pouch round the egg is governed by the amount of surface with which the base of the shell layer is in contact during oviposition, and that this governs the extent of the outer wax, and cement layers. The degree of protection which the outer wax layer confers on some of these eggs must be very much greater than that which it confers on the normal egg.

Discussion

A small egg such as that of the red spider mite must overcome the difficulty of conserving water. Its surface area is huge compared with its water content, and it must, virtually, have a shell which is *impermeable* to water if such a thing can exist. It is not surprising that the shells of the summer egg, lasting 12 days from oviposition, and the winter egg, which may last as much as 6 months in the field (and under experimental conditions may be viable after a year, following suitable temperature treatment) have essentially the same shells—12 days or 12 months are equally large spaces of time if any water loss *at all* is to take place from the egg. The mite has evolved a shell which will completely prevent desiccation, and as a result, apart from the small variation which occurs in the timing of laying down the inner wax layer, it is not unreasonable to suppose that the same system is used in both types of egg. Whether any other form of egg-waterproofing or protecting measures are taken during the life of the summer embryo, or for the protection of the overwintering embryo in diapause, has not been determined in this study. Such additional systems have been found in insect eggs, such as those of *Rhodnius* (Beament, 1949)

and *Melanoplus* (Slifer, 1937), and the results of applied and field workers seem to indicate that changes in resistance to ovicides in other eggs are indications of changes in internal membranes. It is hoped later to study any changes in the shell membranes during development and their possible effect on the variable resistance of the egg.

The mechanism whereby the female produces a winter egg already waterproofed whereas the summer egg is not so equipped, is an obvious starting point for work on the changes which initiate the formation of a diapausing egg rather than a summer type egg.

The subject of fertilization may be considered briefly; the observations are deductive, rather than of an experimental nature. The shell has no micropyle or other form of entrance for sperm. It is very unlikely that the sperm would be able to penetrate the shell layer by chemical activity, seeing the chemical constitution of this material, and one must conclude that the egg is fertilized before the shell layer is produced, i.e. in the ovary before the ovum is completely mature. No sperm, identifiable as such, was seen in the genital canal or ovary, and no conclusions can be made as to the actual time of fertilization. It is, however, probably a similar phenomenon to fertilization in ticks (Lees & Beament, 1948) where the eggs are fertilized precociously before they are even a quarter of their final diameter. The early stage of copulation (immediately on emergence of the female from the last nymphal moult) and the fact that sperm were not found in the spermathecae support this argument.

Several workers (Wigglesworth, 1945, 1947; Beament, 1945, 1946*b*, 1949; Lees & Beament, 1948, etc.) have wondered how a very hard wax in a mobile form rapidly sets to a solid with high melting-point. In the egg of the red spider mite, this wax has very unusual properties, and its function in relation to the egg must surely go beyond the simple water-protecting value which is so obviously defeated by the hole at the base of the egg.

I am much indebted to Messrs M. D. Austin and T. Beament, and to Dr A. M. Massee, who have from time to time supplied material from the field; to Dr V. B. Wigglesworth, F.R.S., and members of the East Malling Research Station for the many useful discussions during this work, and particularly to Dr A. D. Lees, who has been chiefly responsible for the maintenance of cultures from which material for this work was drawn.

REFERENCES

Austin, M. D. & Massee, A. M. (1947). Investigations on the control of the fruit tree red spider mite (*Metatetranychus ulmi* Koch) during the dormant season. *J. Pomol.* **23**, 227.

Beament, J. W. L. (1945). The cuticular lipoids of insects. *J. exp. Biol.* **21**, 115.

Beament, J. W. L. (1946*a*). The formation and structure of the chorion of the egg in an hemipteron, *Rhodnius prolixus*. *Quart. J. micr. Sci.* **87**, 393.

Beament, J. W. L. (1946*b*). The waterproofing process in eggs of *Rhodnius prolixus* Stal. *Proc. Roy. Soc.* B, **133**, 407.

Beament, J. W. L. (1948). The penetration of insect eggshells. I. Penetration of the chorion of *Rhodnius prolixus* Stål. *Bull. ent. Res.* **39**, 359.

Beament, J. W. L. (1949). The penetration of insect eggshells. II. The properties and permeability of the sub-chorial membranes during development of *Rhodnius prolixus* Stål. *Bull. ent. Res.* **39**, 467.

Blair, K. (1949). Unpublished observations.

Blauvelt, W. E. (1945). The internal morphology of the common red spider mite, *Tetranychus telarius*, Linn. *Mem. Cornell agric. Exp. Sta.* no. 270.

Bradfield, J. R. G. (1948). Unpublished observations.

Cagle, L. B. (1946). The life history of the red spider mite. *Tech. Bull. Virginia agric. Exp. Sta.* no. 98.

Davies, Lewis (1948). Laboratory studies on the egg of the blowfly, *Lucilia sericata* (Mg.). *J. exp. Biol.* **25**, 71.

Hurst, H. (1948). Asymmetrical behaviour of insect cuticle in relation to water permeability. *Disc. Faraday Soc.* no. 3.

Jahn, T. L. (1935). The nature and permeability of grasshopper egg membranes. II. Chemical composition of membranes. *Proc. Soc. exp. Biol., N.Y.*, **33**, 159.

Korschelt, E. (1887). Zur Bildung der Eihüllen der Mikropylen und Choriononhünge die den Insecten. *Nova Acta Leop. Carol. D.A. Nat.* **51**, 183.

Lees, A. D. (1950). Diapause and photoperiodicity in the egg of the fruit tree red spider mite, *Metatetranychus ulmi*, Koch. (In the Press.)

Lees, A. D. & Beament, J. W. L. (1948). An egg waxing organ in ticks. *Quart. J. micr. Sci.* **89**, 291.

McDonnough, J. (1909). Ueber den Bau des Darms und seiner Anhänge von *Chrysopa perla* L. *Arch. Naturgesch.* **75**, 313.

Massee, A. M. (1947). A new method of rearing the fruit tree red spider mite (*Oligonychus ulmi* Koch) on apple seedlings. *Rep. E. Malling Res. Sta.* 1946, p. 122.

Michaelis, L. & Goddard, D. R. (1934). A study on keratin. *J. biol. Chem.* **106**, 605.

Newcomer, E. J. & Yothers, M. A. (1929). Biology of the European red mite in the Pacific North-West. *Tech. Bull. U.S. Dep. Agric.* no. 89.

Nielsen, E. T. & Thamdrup, H. M. (1939). Ein Hygrometer für mikroklimatische Untersuchungen. *Bioklim. Beibl.* **4**, 180.

Ramsay, J. A., Butler, C. G. & Sang, J. H. (1938). The humidity gradient of the surface of a transpiring leaf. *J. exp. Biol.* **15**, 255.

Slifer, E. H. (1937). The origin and fate of the membranes surrounding the grasshopper egg. *Quart. J. micr. Sci.* **79**, 493.

Steer, W. (1940). Migration of the red spider. *Gard. Chron.* **108**, 28.

Theobald, F. V. (1911). Report on economic zoology. *J. S. E. agric. Coll. Wye*, **20**, 120.

Tichomiroff, A. (1885). Chemische Studien über die Entwicklung der Insecteneier. *Z. physiol. Chem.* **9**, 518.

Verson, E. (1884). Sulle uova dell' insetto (*Bombyx mori*). *Boll. mens. bachicoltura Padova*, no. 9.

Wigglesworth, V. B. (1945). Transpiration through the cuticle of insects. *J. exp. Biol.* **21**, 97.

Wigglesworth, V. B. (1947). The epicuticle of an insect, *Rhodnius prolixus*. *Proc. Roy. Soc.* B, **134**, 163.

(*Received* 26 *June* 1950)

HOST ALTERNATION IN *APHIS FABAE* SCOP.

I. FEEDING PREFERENCES AND FECUNDITY IN RELATION TO THE AGE AND KIND OF LEAVES

By J. S. KENNEDY and C. O. BOOTH

Agricultural Research Council, Unit of Insect Physiology, Zoology Department, Cambridge

(With 7 Text-figures)

The feeding preferences and comparative fecundity of laboratory-bred, alienicolae alatae of *Aphis fabae* were investigated in small leaf cages on spindle and sugar beet leaves, representing a winter and a summer host respectively, both in pots in the greenhouse and while growing naturally outdoors.

The aphids' readiness to stay and feed, and their average reproduction rate, both varied between leaves of different ages and kinds, and there was evidence of true fecundity differences among aphids feeding on different leaves. As a general rule, readiness to settle and reproduction rate varied together in the same sense, but they did so significantly less often in comparisons between leaves of different kinds, spindle and beet, than in comparisons between leaves of one kind only. Among the leaves on the same kind of plant, the aphids preferred to feed and reproduced faster, on the whole, on young and early senescent leaves than on mature ones. To a limited extent, this rule seemed to govern also the aphids' comparative readiness to settle and reproduce, as between leaves of the two different kinds of plant. Allowing for age-differences among the leaves, the aphids settled and reproduced better on spindle than on beet leaves. Since the aphids used were alienicolae, this tends to confirm the usual designation of the winter host as primary and the summer hosts as secondary, for a given aphid species.

These findings form the basis of a dual discrimination theory of host selection, which assumes that aphids respond behaviourally to at least two main classes of leaf property: one associated with the age of the leaf and the other with the kind of plant. It is suggested that in nature these two sensory requirements of the aphids may be essentially contradictory, and that the shifting patterns of aphid distribution among leaves and plants may depend on the shifting distribution of leaves offering a satisfactory compromise between them. The phenomenon of host alternation is considered in the light of the dual discrimination theory, as a particular instance of a shifting distribution-pattern probably connected with the alternation of the seasons of active growth and senescence in the winter and summer host plants.

Introduction

Aphis fabae, like many other aphid species, shows a seasonal alternation in the types of host-plant it colonizes, being found on certain shrubs in the spring and autumn (over-wintering there in the egg), and on various herbs* in the summer (Davidson,

* The terms shrub and herb will be used in this paper in the sense implied by Strasburger (1930, p. 134): the aerial parts of a shrub are perennial, whereas those of a herb are annual. Herbs include both summer annuals in which the winter is passed as the seed, and winter annuals in which the summer is passed as the seed.

1921; Mordwilko, 1936; Jones, 1947). In the spring, the spindle tree (*Euonymus europaeus* L.) and to a lesser extent certain other shrubs, sustain colonies of apterous fundatrices, fundatrigeniae and their offspring with developing wings. In the autumn, these shrubs are infested by gynoparae and sexuales. In the summer, the same aphid species infests beans and other herbs, in the form of winged and wingless alienicolae.

Two hypotheses suggest themselves in approaching this problem. The first concerns seasonal changes in the aphids themselves. Since the various aphid forms differ in structure and sometimes also in reproductive physiology, they might well differ also in behaviour and nutritional physiology, that is in the particular plant species on which they will settle and reproduce. Thus the virginoparous spring and summer migrants might prefer beans, sugar beet, etc., to spindle, while the gynoparous and male autumn migrants might prefer spindle to the summer host plants. This hypothesis of a change in the aphids' preferences as the cause of their change of hosts, has been stated explicitly by Dethier (1947, p. 255), and is clearly implicit in this passage from Hille Ris Lambers (1945, p. 69):

> Those [alatae] of the second generation are supposed to be incapable of producing larvae on apple because species with a tendency toward migration cannot use their spring migrants for colonization on winter hosts.

The second hypothesis concerns seasonal changes in the host plants. It was implicit in Davidson's statement (1921, p. 87):

> In my experiments it was found that the alienicolae alatae of any generation could be transferred back to *Euonymus*, on which plant they produced young, and eventually in succeeding generations both sexual ♂♂ and sexual ♀♀ appeared.... *It should be noted, however, that young growth was ensured on the* Euonymus *bushes by cutting them back.* [Our italics.—J.S.K. & C.O.B.]

Certain observations of Mordwilko (1907) were cited by Davidson (1921) in support of his suggestion that 'in the adventures of migration the alienicolae alatae of any generation may alight on the winter host and produce young'. Mordwilko (1936) later produced further field and experimental evidence of the readiness of alienicolae to colonize winter hosts and, less certainly, of fundatrices from spindle accepting a summer host. There is doubt about the identity of some of the aphids studied by Davidson and Mordwilko, for the systematics of the black aphids are even now not fully worked out (Jacob, 1945, 1949); but that does not invalidate their observations for present purposes.

Mordwilko (1936) attributed the normal failure of alienicolae to colonize spindle, to the robust condition of its leaves when exposed to the summer sun, since he found that colonies did develop on soft, shaded growth. He therefore described migration as *facultative* in *A. fabae*. The term migration is here made synonymous with heteroecy (host alternation), a confusing usage which has unfortunately gained currency (e.g. Hille Ris Lambers, 1934). Migration in the literal sense of persistent, spontaneous flying, is also a facultative, not an obligatory phase in the life of the *A. fabae* alata before it settles down to breed (Kennedy & Booth, 1950, p. 466). And when such migration does occur, it is a separate question whether the migrants

colonize the same kind of plant as that on which they developed, or plants of a different kind. Mordwilko's finding in this connexion, that *A. fabae*'s habit of colonizing a different kind of plant after the spring migration, is facultative, being necessary only because the winter host normally passes into an unsuitable condition in summer, provides clear evidence that host alternation may be due to changes in the plants.

Mordwilko's and Davidson's work did not, however, provide any obvious explanation for the abandonment of summer hosts and successful colonization of spindle in the autumn. Davidson (1921, p. 88) remarked that at this time the summer hosts became unfavourable whereas the winter hosts then offered the most favourable food conditions. But it was not clear why this should be so, for the return migrants colonize the old leaves, young growth being very scarce at this time. And although the change in the weather in autumn is toward conditions approximating to those under shade in summer, this external change is the same for both summer and winter hosts.

Thus while there is more evidence for the second hypothesis of host alternation than for the first, neither has been fully tested and the issue remains in doubt. A more complete explanation of host alternation in terms of the second hypothesis was suggested by observations on the distribution of aphid infestations on growing plants (Kennedy, Ibbotson & Booth, 1950; Ibbotson & Kennedy, 1950). Spindle and sugar beet leaves showed *two* periods of high susceptibility to *A. fabae* (and to *Myzus persicae* (Sulz.)), one when they were growing and the other when they were beginning to senesce. Alternating with those periods were two periods of low susceptibility, one at maturity and the other in late senescence. It follows that the susceptibility of a plant, considered as a whole relative to other whole plants, will depend on, among other things, what we may call the *leaf age-composition* of the plant: that is, the amount of growth and senescence going on among its total complement of leaves. The leaf age-composition changes as the plant develops. Host alternation seems likely to depend on these seasonal changes in the plants because the seasonal cycle of leaf age-composition of the winter host appears to complement that of the summer hosts, from the aphids' point of view. The winter host has a cycle typical of shrubs and trees, with a period of mass growth in the spring and a period of mass senescence in the autumn. During the intervening summer period, the shrub gives the impression of being relatively static, with most of its leaves mature, but that is the time when the summer hosts, being herbs, are engaged in active, simultaneous growth and senescence.

The present paper deals with experimental work on the second hypothesis of host alternation. The hypothesis requires that forms of aphid not usually found either on summer hosts, or on winter hosts, should nevertheless be able to colonize them in appropriate circumstances, and there is already some field evidence of this (Davidson, Mordwilko, and Kennedy *et al.* loc. cit.). The field evidence is limited, however, because each aphid form is present in nature only at certain seasons and does not have the opportunity of colonizing the various host plants at all stages of their

development. Host transference experiments with one form of aphid were therefore conducted systematically throughout the year, outdoors or in the greenhouse, in order to explore the effect of leaf age on host susceptibility. At the same time the experiments were designed to show how far the aphid's preferences in the strict behavioural sense, as distinct from variations in their fecundity reflecting varying nutrition, were responsible for variations in the weight of infestation on different plants or parts of plants.

Material

The aphids used were 12–48-hr.-old *virginoparae alienicolae* from a clone of *Aphis fabae* which was started with an apterous viviparous female taken from sugar beet in Cambridge in October 1946. The aphids were reared on broad beans (*Vicia faba* L.) and the alate and apterous summer forms were produced *en masse* daily throughout the year as described by Kennedy & Booth (1950).

The plants used were the spindle tree (*Euonymus europaeus* L.) as *Aphis fabae*'s main winter host, and sugar beet (*Beta vulgaris* L.). Sugar beet was chosen as an important crop plant to which this aphid causes losses both directly and indirectly as a virus vector. Sugar beet also met the requirement of a summer host normally present throughout the period when the winter host is in leaf. The over-wintered plants of the beet seed crop are overlapped in time by the plants of the root crop (some of which are not lifted till after spindle leaf-fall), and the root crop plants are overlapped in their turn by the young plants of the next year's seed crop. The beet has conveniently large, strong leaves, often present in all stages of growth and senescence on the same plant. Finally, beets (like spindle trees) are botanically unrelated to the beans used for rearing the experimental aphids, so that any conditioning effect of the previous host plant upon the preferences of these aphids, which might have influenced the results of host-transference experiments, was at any rate standardized.

A few of the earlier spindle trees used in pots were lifted from the grounds of the Entomological Field Station, Cambridge, but most of the pot plants and all of the spindle trees used outdoors were taken from a coppice on White Hill, 3 miles south of Cambridge. Persistent variations were noticed in the shape and size of the leaves, the general habit of growth, colouring of the stems, etc., among the spindle trees used. One specimen was identified as var. *intermedia* Gaud., the others as apparently typical. We know of no evidence that varieties of *Euonymus europaeus* differ in their susceptibility to *Aphis fabae*.

For both pot work and planting in the raised bed young beet plants of Bush E variety (a mixture of nine strains) and seed of the same variety (but of one strain) were used. Both plants and seed were kindly provided by Dr S. Ellerton of British Pedigree Sugar Beet Seed Ltd., Maldon, Essex.

Most of the indoor and outdoor experimental beets sooner or later showed symptoms of virus diseases: yellows or mosaic, or both. Plants with obvious symptoms were generally rejected for indoor experiments and plants with severe symptoms

were rejected outdoors, but toward the end of their growing seasons practically all the outdoor experimental plants were showing some symptoms.

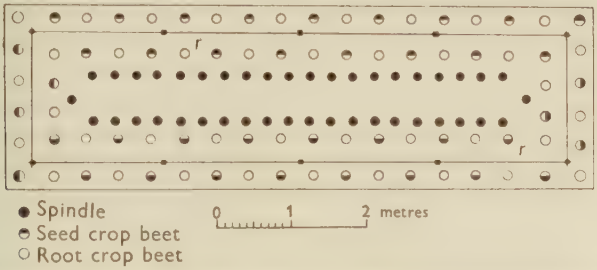


Fig. 1. Diagrammatic plan of raised bed used for outdoor experiments on spindles and beets grown in their natural seasons. *r*, rail supported on stakes for attachment of leaf cages.

Method

Preparation of plants

The spindle and beet plants for the main series of experiments were to be grown in the existing soil outdoors, so that their acceptability to the laboratory bred, summer form of aphid could be compared at intervals throughout their natural cycles of growth, maturation and senescence. The spindles and beets were therefore planted in parallel rows in a raised bed in the garden, so that the spindle leaves could be brought into position for caging together with the beet leaves, and the fatigue of repeated stooping was minimized.

The retaining walls of the raised bed (Figs. 1 and 2) were constructed and filled with previously manured top-soil in December 1947. In February 1948, young beets and dormant spindle trees were lifted from reserve beds and planted in the raised bed in the pattern shown in Fig. 1. The young spindle plants in the raised bed had been selected for their long slender stems so that they could be bent down for their leaves to be caged alongside beet leaves. The supporting arms of the leaf cages were clamped where required on the iron rail shown in Figs. 1 and 2.

In April, seed for the root crop was sown in the spaces between the now actively growing seed-crop plants. The root-crop seedlings were later thinned to leave only one in each position shown in Fig. 1. The seed-crop plants were 'topped' in the usual way and finally pulled out in July, leaving the root crop plants for further comparison experiments with the spindle leaves, until these leaves fell and the beets had ceased growth in November.

In addition to these spindles and beets allowed to grow outdoors in their normal season, others were lifted from the reserve beds from time to time and potted up for greenhouse experiments, growth and senescence being induced artificially when necessary. They were potted in John Innes potting compost (Lawrence & Newall, 1948).

No special treatments were required to obtain sugar-beet leaves of any desired age even in the winter, for once active vegetative growth started in the greenhouse, leaves of all ages were soon available on the same plants. A difficulty with the beets, which arose only when the original practice of bringing fresh plants into the greenhouse at frequent intervals was discontinued, was to prevent bolting under the 16 hr. light régime of the experimental house. Bolting was delayed to some extent by the high temperature (about 20° C.) maintained to ensure a satisfactory level of activity among the summer-type aphids used for all the experiments. On the other hand the warmth led to rather soft, drawn growth by both beets and spindles, especially under the restricted illumination of winter. As far as possible plants not in immediate use were kept in a cooler house in winter, and outdoors in summer.

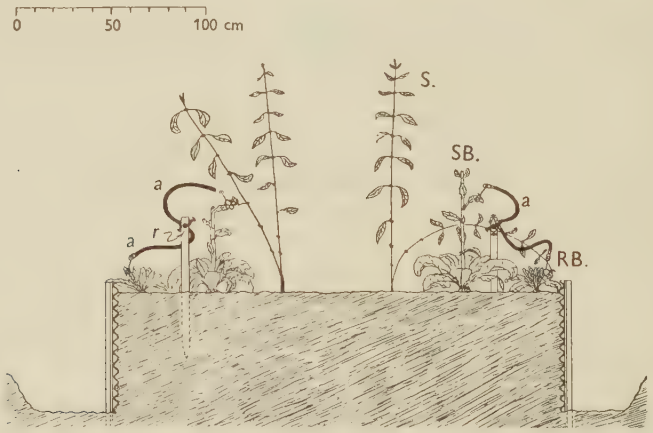


Fig. 2. Cross-section of raised bed, showing *S*, spindle; *SB*, seed-crop beet; *RB*, root-crop beet; and leaf cages in position with their supporting arms *aa* clamped to the fixed rail *r*.

The spindles, which do not normally bear leaves of all ages at the same time, responded well to simple treatments for the induction of out-of-season growth and senescence. In the winter and spring, dormant plants began growth almost at once on being brought in from outdoors to a house at about 10° C. After a week they were transferred to a house at about 15° C. and after a further week to the experimental house. They bore leaves large enough for the experimental cages in about 3 weeks from bud-break and had ceased growth in about 2 months. New young growth was obtained also by pruning pot plants in full leaf, but such growth was not as vigorous as that from dormant plants.

Senescence, leading eventually to leaf-fall and dormancy, was induced in mature spindle trees in various ways: by heavy watering combined with passage through successively cooler greenhouses; by transfer to a room at a temperature of 13–15° C.,

with dim light (150 m.c.) for 8 hr. daily, without changing the water-supply rate; or by denying the plants water for a while and then resuming watering without changing the temperature and light treatments. In the early summer, senescent leaves were available on plants forced indoors in the winter. Later in the summer, they were made available by transferring outdoor-grown plants with mature leaves to the cool, dimly lit room. New growth was obtained in the summer by bringing out from a cold store dormant plants placed there the previous winter. Such plants did not remain dormant indefinitely even in the cold store, and in the late summer new growth was obtained on plants which had been forced to senesce and drop their leaves in the summer.

By such means spindle leaves of different ages, young and mature, mature and senescent, and even all three together on the same plant, were made available at the same time for comparison one with another and with the sugar-beet leaves.

Recording of leaf growth and senescence

The *stage* reached by the experimental leaves in the process of growth and senescence, was recorded on an arbitrary scale of ten types of leaf distinguishable by their external appearance. The main criteria of type were the degree of unrolling and the colouring of the leaf, but position and size in relation to the other leaves on that shoot or plant were also taken into account. This typing system has been described more fully elsewhere (Kennedy, Ibbotson & Booth, 1950), and may be summarized as follows:

Type symbol	Brief description
YY	Starting to unfurl.
Y	Not quite fully unfurled.
YM	Unfurled, but paler than the darkest green leaves present.
M	The darkest green and most glossy leaves on the plant.
M(O)	Similar to *M* but less glossy and with a faint yellowish tinge.
MO	Definite yellowing of up to 10% of the blade area.
O_1	10–25% yellowing.
O_2	25–50% yellowing.
O_3	50–75% yellowing.
O_4	75–100% yellowing.

The *rate* at which leaves were growing and senescing was estimated by recording their types and areas at the beginning and again at the end of each experiment, covering a period of one day for indoor experiments, and 3–5 days outdoors. The area of a leaf, required only for comparative purposes, was taken as the product of length and breadth. A spot of cellulose paint at the junction of lamina and petiole served as a fixed point from which the length was measured. The growth-rates quoted in the tables below are calculated from

$$\frac{100 \times (A_2 - A_1)}{A_1 \times D},$$

where A_1 = initial area, A_2 = final area, D = number of days between area measurements. Thus the figures are average daily percentage increases in area.

There was a good correlation between type and area growth-rate among the young leaves actually used, although not, of course, among all young leaves. Thus the *YY*, *Y* and *YM* types usually showed growth-rates of 10 or more, between 1 and 10 and less than 1%, respectively, for spindle; and of 20 or more, between 10 and 20 and less than 10%, respectively for beet. When therefore there was doubt over the correct typing of a leaf, and its growth-rate was known, this was taken into account in assigning the leaf to a type.

Experimental procedures

The modified host-transference techniques used in these experiments are described in a separate paper (Kennedy & Booth, 1950), to which reference should be made for details concerning not only the methods themselves but also the interpretation of the results they give. The host plants were presented to the aphids in a standardized manner, as small areas of leaf undersurface. The experiments were of two types, preference experiments and reproduction experiments, often run concurrently on the same set of plants. In every experiment, two or more different types of leaves were compared, each type being represented by several separate leaves.

In the preference experiments, equal areas of two different types of leaves were enclosed in the same cage and counts were taken of the distribution of feeding aphids between the two leaves. The standard procedure in most of the indoor experiments and all of the outdoor experiments was to make four counts at 20- to 30-min. intervals from the time of introducing the aphids into the cages. If the average majority of aphids counted on one leaf was greater than the square root of the average total on both leaves, the result was classed as a *win* for that leaf. If not, the result was classed as a *draw* (Kennedy & Booth, loc. cit.). Alternatively, results were expressed as percentage distributions, or simple majorities, where this gave a more sensitive measure of preferences.

In the reproduction experiments, leaves were caged singly and counts were taken of the parent aphids found feeding, wandering and dead, and of the young they bore. The counts were taken at appropriate intervals, and the results were worked out, as indicated below in the descriptions of the various experiments.

Results

Summer aphids and the winter host: preliminary experiments

In the first series of greenhouse experiments, ten alate and ten apterous adults were introduced together into preference cages enclosing young growing spindle leaves alongside mature leaves of seedling sugar beets (six-leaf stage). In all, twenty-two separate pairs of such leaves were caged for comparison. Two cages were stocked twice in succession without changing the leaves and the others once each, and a single distribution count was taken some hours after each introduction. Given this large number of cagings each of a small number of aphids, the preference is most clearly

shown by dividing the results into those in which a simple majority of aphids was found on the spindle leaf, those with a simple majority on the beet leaf, and those with equal numbers on the two leaves. On that basis, both aphid forms showed (Table 1) a clear preference for spindle.

TABLE 1. *Preference experiments (pot plants): young spindle compared with mature beet leaves (January 1948)*

Aphid form	Majority settled on: Spindle	Majority settled on: Beet	Equally distributed	Total presentations
Alate	21	2	1	24
Apterous	18	4	2	24

TABLE 2. *Preference experiments (pot plants): apterae and their young settled on young spindle leaves as compared with mature beet leaves (January 1948)*

Cage	Adults: Total both leaves	Adults: % on spindle	Nymphs: Total both leaves	Nymphs: % on spindle
1	17	94	117	73
2	14	78	115	87
3	16	81	170	88
4	9	78	90	78
5	10	70	100	90
Means	—	80	—	83

Concurrently, five preference cages containing similar pairs of leaves were stocked once each with thirty to thirty-five apterous adults, which were left to bear young overnight. The results (Table 2) are given as percentages of adults and nymphs found settled on the two leaves, for in these experiments there was a small number of admittances and a relatively large number of aphids. Both mothers and nymphs were found settled in a substantial majority on the spindle leaf in every experiment.

Since adult aphids move about more than young nymphs do (Ibbotson & Kennedy, 1951), it is probable that the occurrence of more nymphs on the spindle than on the beet leaves can be attributed, in the main, simply to the settling of more adults on the spindle leaves. But the freedom for both adults and nymphs to move to and fro between the two leaves makes it impossible to judge whether the preponderance of adults on the spindle alone explains the preponderance of nymphs there, or whether, in addition, the individual mothers produced more (or less) young while settled on the spindle than on the beet leaves. It was for this reason that the use of these two-leaf cages for preference measurement was later supplemented by the use of single-leaf cages for the measurement of comparative reproduction rates.

In the third type of preliminary experiment, young spindle leaves were compared with young beet, and with mature spindle leaves. Five similar, growing leaves were selected from one vigorously sprouting pot spindle; three were caged alongside

TABLE 3. *Preference experiments (pot plants): alatae settled on young spindle as compared with mature spindle leaves, and as compared with young beet leaves (March, 1948)*

Spindle *v.* spindle		Spindle *v.* beet	
Total	% on young spindle	Total	% on spindle
48	69	50	46
20	90	36	56
29	84	54	54
27	74	24	88
—	—	51	65
—	—	16	81
Means	79	—	65

rapidly growing young beet leaves and two alongside mature leaves on another pot spindle which had largely ceased growth. The cages were stocked with twenty alatae each, and four counts of the aphids settled on the leaves were made at half-hourly intervals after loading. The cages were restocked and fresh counts made next day, which provided a total of four infestations of the young-mature spindle cages and six of the young spindle-young beet cages. For each presentation, the sum of the four counts on the young spindle leaf is given in Table 3 as a percentage of the combined sum for both leaves in each cage. It will be seen that the aphids preferred the young spindle both to the mature spindle and to the young beet leaves. Moreover, 69% or more of the aphids were found on the young spindle leaf in all the four cases when the alternative was a mature spindle leaf, whereas 65% or less were found on the young spindle in four of the six cases when the alternative was a young beet leaf. The aphids seemed to discriminate more sharply between the two ages of leaf on one kind of plant, than they did between the two kinds of plant.

These preliminary experiments showed that the summer form of the aphid has no fixed preference for a summer host over a winter host, considered as plant *species*. Under certain conditions the summer form of aphid actually prefers the winter host. The experiments suggested that the principle derived from the study of free infestations on single plants—that young and senescent leaves are more susceptible to aphids than mature leaves—might be extended to the relations between different plant species and so explain the changing distribution of the aphids among their host species in nature.

The preliminary experiments suggested that one form of aphid could be used to investigate the effects of leaf age on both spindle and sugar beet, thus avoiding for the time being the complication that different aphid forms might have different preferences. Of the two forms that could be produced easily all the year round (Kennedy & Booth, 1950)—winged and wingless *virginoparae alienicolae*—the winged form was chosen for most of this work because, in nature, it has better

opportunities for sampling different hosts and must play the greater part in selection between plant species.

TABLE 4. *Preference experiments (pot plants):* wins *gained by young spindle* v. *mature spindle leaves*

Date	Cages	Presentations	Wins *Y*	Wins *M*
March 1948	4	4	2	0
November 1948	2	12	6	1
March 1949	4	12	6	0
March 1949	4	12	4	1
Totals	14	40	18	2

Feeding preferences between leaves of the same plant

Direct comparisons in the greenhouse

Spindle. The results of four further greenhouse experiments in which young and mature leaves on similar, well-watered pot spindle plants were compared with each other in preference cages, on various dates, are summarized in Table 4 in terms of the number of *wins* gained in each leaf type. Half the presentations resulted in *draws* but eighteen out of the total of twenty *wins* were gained by the young leaves, which showed their superiority in every experiment. Table 5 contains the results of a compound preference experiment in which several different types of spindle leaves were compared with one another at the same time. The *Y* and *YM* leaves used were selected from among the leaves on one pot spindle, the *M* leaves from another plant and the senescent leaves from a third. There were two cages from each pair of leaf types to be compared, and each cage was stocked with thirty alatae nine times, making a total of eighteen presentations for each class of comparison. The leaf-type symbol 'O_1' refers here to leaves which in fact ranged in type from *MO* to O_2.

TABLE 5. *Compound preference experiment (pot plants):* wins *gained by spindle leaves of various ages when compared with one another (November* 1948)

Comparison	*Wins*	
Y v. *M*	*Y* 13	*M* 1
O_1 v. *M*	O_1 7	*M* 3
Y v. *YM*	*Y* 4	*YM* 2
YM v. *M*	*YM* 6	*M* 2
Y v. O_1	*Y* 7	O_1 3

According to the first two lines of Table 5 the mature leaves were inferior to both young and senescent leaves. Lines 3 and 4 of the Table suggest that leaves become progressively less acceptable as their growth slows down, *Y* leaves being superior to *YM* leaves which are in turn superior to *M*. Although some of the preferences are not very striking, their significance is increased by the self-consistency of the results

as a whole. Thus the preferences between *Y* and *YM*, and between *YM* and *M*, are in the same direction as, but weaker than, the preference between *Y* and *M*; and the weaker preference of O_1 over *M*, as compared with that for *Y* over *M*, suggests a superiority of *Y* over O_1 which is indicated directly in the last line of Table 5.

TABLE 6. *Compound preference experiment* (*pot plants*): *spindle leaves of various ages on the same shoots* (*lot A*) *compared with a control lot* (*B*) *of mature spindle leaves* (*March* 1949)

	Wins by leaves		Simple majorities on leaves	
Lot A leaves	Lot A	Lot B	Lot A	Lot B
Young	7	0	9	0
Mature	0	1	4	5
Senescent	3	0	9	0

A further compound preference experiment was designed to control the possibility of there being invisible differences between leaves of the same apparent age on different plants, or on the same plant, which might interfere with the comparison of leaves of visibly different ages. Nine leaves, forming lot A, comprised three young, three mature and three senescent (types O_1 and O_2) leaves borne on two shoots of one pot spindle and one shoot of another. The shoots were still growing and offered a complete range of leaf ages from young to senescent between tip and base. One young, one mature and one senescent leaf were selected on each shoot. Nine mature leaves, as alike as possible, formed lot B for use as controls, and were selected from among the leaves on three other pot spindles. Nine double-leaf cages were set up, each containing one leaf from lot A and one from lot B, and the cages were loaded three times. Table 6 shows that the mature leaves of lots A and B were about equally attractive to the aphids, whereas there was always a majority of aphids found settled on the young and senescent leaves of lot A as compared with the mature leaves of lot B. Judging by the numbers of *wins*, the young leaves were, as before, more attractive than the senescent ones.

TABLE 7. *Compound preference experiment: as Table* 6, *repeated* (*April* 1949)

	Wins by leaves		Simple majorities on leaves	
Lot A leaves	Lot A	Lot B	Lot A	Lot B
Young	6	0	11	1
Mature	0	0	8	3
Senescent	0	0	10	1

The same experiment was repeated a few days later, when each cage was stocked four times, giving a total of twelve presentations for each class of comparison. New young and senescent leaves were chosen on the same shoots as before, since the previous ones had by now become too old. The results (Table 7) fell into the same general pattern as before, with young and senescent leaves proving superior to

mature ones, and young leaves superior to senescent ones. But the superiority of senescent over mature leaves on the same shoot was less marked than before, because this time even the mature leaves of lot A proved superior to the lot B leaves. This may have been due to the lot A plants being rather more turgid than lot B, a point to be discussed in a later paper.

TABLE 8. *Compound preference experiment (pot plants): mature spindle leaves compared with senescing spindle leaves of various ages (February* 1948)

		Adults		Young	
Comparison	Cage no.	Total settled	% settled on *M* leaf	Total settled	% settled on *M* leaf
M v. *MO*	1	14	7	15	20
	2	15	26	19	21
M v. O_2	3	19	32	13	70
	4	20	45	21	38
M v. O_4	5	16	100	7	100
	6	20	100	8	100

One experiment was done on spindle leaves forced to senesce rapidly by detaching shoots from the plant. Shoots that had ceased growth and bore six to ten pairs of mature leaves each, were cut off a large pot spindle and kept in water in semi-darkness at 25° C. for a week. At the end of that time the leaves ranged in colour from pale green (*MO*) at the tip to completely yellow (O_4) at the base of the shoot, and the lowest leaves were falling. An *MO*, an O_2 and an O_4 leaf on each of two shoots were caged alongside mature leaves on shoots still attached to the original pot plant in the greenhouse, and each cage was stocked once with twenty alatae. The distribution of settled parents was recorded at 3 hr. and that of their young at 24 hr. after loading. The results are shown in full in Table 8. The distribution of both parents and young indicate that the *M* leaves were less attractive than the early senescent leaves *MO*, slightly less attractive than or about equal to the later senescent leaves O_2, and much more attractive than the oldest leaves O_4.

Beet. A greenhouse experiment using eight cages each stocked once with thirty alatae, and in which young growing leaves were compared with mature leaves of the same batch of pot beets, gave six *wins* for the young leaves, one for the mature leaf and one *draw*. Next morning thirty-three young were found on all the young leaves and only four on the mature leaves. But the bulk of the information on settling preferences as between beet leaves was obtained from indirect comparison experiments.

Indirect comparisons outdoors

The preference experiments on the spindles and beets growing naturally outdoors in the raised bed (p. 29) and extending throughout the life of the spindle foliage are listed in Tables 9 and 10. Exp. O was carried out in the greenhouse, but on

plants grown outdoors in their natural season. The beet leaves used were on a plant that was lifted and potted with a large mass of undisturbed soil (60 cm. deep and 40 cm. across) enclosing its roots. The spindle leaves were on plants grown in large pots which had been left sunk in the ground. Both were brought into the greenhouse immediately before the experiment, and kept watered. The object of bringing the plants indoors was to keep the aphids warm so that they would move about and reproduce more than they were able to do outdoors in the cold dull weather of most of October 1948.

TABLE 9. *Summary of outdoor preference experiments comparing spindle leaves with seed-crop sugar-beet leaves (except for Y and M leaves in Exp. F1, which were on root-crop beets) (April–July 1948)*

		Leaves compared							
		Spindle		Beet			Total	*Wins*	
Exp.	Date	Type	Growth rate %	Type	Growth rate (%)	No. cages	present-ations	Spindle	Beet
A	5–7 Apr.	*YY*	16	*Y*	11	3	5	5	0 α
		Y	4	*YY*	21	5	13	5	5 β
B	20–21 Apr.	*Y*	6	*YY*	33	3	6	1	1
		Y	7	O_1–?	1	4	8	3	2
B1	22–23 Apr.	*Y*	4	*YY*	39	4	8	6	0
		Y	4	O_1–?	1	4	8	5	1
C	27–29 Apr.	*Y*	6	*Y*	11	3	7	5	1
		Y	9	O_1–?	0	3	7	4	1
D	6–7 May	*Y*	3	*Y*	17	6	12	4	7
D1	11–12 May	*Y*	4	*YY*	24	3	6	5	0, α
		YM	1	*YY*	35	3	6	0	4, β
E	20–21 May	*Y*	5	*Y*	12	3	6	6	0, α
		YM	0·5	*YY*	20	3	6	0	3, β
F1	18 June	*M*	0	*Y*	14	4	8	1	4
		M	0	*M*–?	1	2	4	3	0
		M	0	*MO*–?	0	3	6	2	0
F	23–24 June	*Y*	4	*Y*	14	3	12	5	0
		M	0	*Y*	15	3	12	1	9
		M	0	*M*–?	1	3	12	3	0
G	30 June –1 July	*Y*	6	O_1–O_2	0	2	7	3	0
		M	0	*M*–*M*(*O*)	1	2	7	1	0
		M	0	*MO*–O_1	0	2	7	0	4
		M(*O*)	0	*Y*	17	2	7	0	5
		M(*O*)	0	O_1–O_2	0	2	7	3	0
G1	2 July	*M*	0	*Y*	6	1	2	0	2
		M	0	*M*	0	1	2	2	0

The outdoor experiments (including O) were always done with leaves of the two different host plants caged together, and so did not give a direct comparison of leaves of the same kind of plant. But these experiments were all compound preference experiments so that it was possible to compare different leaves of the same

kind of plant indirectly. Often, leaves of two different ages on one kind of plant were tested in parallel against leaves of one age on the other. The latter then provided a common standard against which the two former could be compared with one another. All such cases have been extracted from Tables 9 and 10 and entered in Tables 11, 12 and 13.

TABLE 10. *Summary of outdoor experiments comparing spindle leaves with root-crop sugar-beet leaves (July–November* 1948)

		Leaves compared							
		Spindle		Beet			Total	*Wins*	
Exp.	Date	Type	Growth rate (%)	Type	Growth rate (%)	No. cages	present-ations	Spindle	Beet
H	7–8 July	*Y*	5	*YY*	23	2	8	4	0
		M	0	*Y*	15	2	8	2	2
		M	0	*M*	0·5	2	8	6	0
		M	0	*MO*	0	2	8	5	0
		M(O)	0	*MO*	0	2	8	6	0
I	28–29 July	*M*	0	*YY*	24	4	16	0	16
		M	0	*M–M(O)*	1	2	8	0	8
		M	0	*M(O)–MO*	0	2	8	1	0
		M(O)	0	*M(O)–MO*	0	2	8	6	0
J	5–6 Aug.	*M*	0	*YY*	23	2	6	2	3
		M	0	*M–M(O)*	1	2	6	2	2
		M	0	*MO–O*$_1$	0	2	6	4	0
		M(O)	0	*YY*	20	2	6	1	3
		M(O)	0	*M–M(O)*	1	2	6	2	1
K	11 and 15 Aug.	*M*	0	*YY*	27	2	8	0	4
		M	0	*M–M(O)*	0·5	2	8	0	0
		M	0	*MO–O*$_1$	0	2	8	0	2
		M(O)	0	*Y*	16	2	8	0	1
		M(O)	0	*M–M(O)*	0	2	8	2	0
L	29–30 Sept.	*M–M(O)*	0	*MO*	0	2	8	2	1
		MO	0	*M*	0	2	8	3	0
		MO	0	*MO*	0	3	12	5	1
M	13 and 15 Oct.	*MO*	0	*M*	2	4	8	3	1
		MO	0	*MO*	0	4	8	0	1
N	20–22 Oct.	*M–M(O)*	0	*MO*	0	4	16	2	5
		MO	0	*MO*	0	4	20	3	6
O	1–4 Nov.	*MO–*?	0	*Y*	?	6	39	16	8
		MO–?	0	*M*	0	3	24	13	1
		MO–?	0	*MO*	0	3	24	10	1

In order to consolidate the data in these tables, leaves that were *YY* or *Y* are all classed as *Y*; leaves that were initially *M* are classed as *M*; and leaves that were initially *M(O)*, *MO* or O_1 are classed as *O*.

Since no two experiments were strictly alike, the whole body of data is best presented by classifying the experiments according to the gross results of each kind of indirect comparison. Of two different leaf types used in parallel tests against a common standard, one would gain a higher proportion of *wins*, out of the total

loadings, than the other; or the proportion of *wins* would be the same for both leaves. That is what is meant by the gross result of a comparison. In Tables 11 and 12, the experiments are sorted into the following classes of gross result, according to whether the proportion of *wins* was:

Greater for young than for mature leaves	$Y > M$
Greater for mature than for young leaves	$Y < M$
Greater for senescent than for mature leaves	$O > M$
Greater for mature than for senescent leaves	$O < M$
Or Equal as between the two leaf types compared	$Y = M$ or $O = M$.

In Table 13, the results are summarized numerically, mainly to show the quantity of readings involved. But the number of loadings and other conditions varied from experiment to experiment so that these combined figures have less significance than the distribution of preferences among individual experiments, shown in Tables 11 and 12 and Fig. 3.

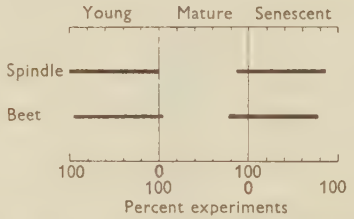


Fig. 3. Summarized results of outdoor preference experiments among leaves of the same kind of plant. Data from Tables 11 and 12. The horizontal blocks extend on each side of the vertical (zero) lines, to a distance representing the percentage of comparison experiments of which the 'gross result' favoured the leaf type indicated.

Considered as a whole, the results adequately substantiate the previous finding that the aphids prefer both growing and senescent leaves to mature ones, of the same kind of plant. At the same time the results give some indication of the limitations of this principle in nature. The results vary considerably between experiments, that is in varying weather, plant and other conditions. Reference back from Tables 11 and 12 to Tables 9 and 10 brings out these variations.

TABLE 11. *Preferences among the leaves of one kind of plant: spindle. Classified results of outdoor experiments in which two types of spindle leaf were compared indirectly in parallel tests against one type of beet leaf.*

Spindle leaves indirectly compared	Beet leaf used as common standard for comparison	Experiments in which gross results showed		
		$Y > M$	$Y = M$	$Y < M$
Young *v.* mature	Young	F, H	—	—
	Senescent	G	—	—
		$O > M$	$O = M$	$O < M$
Mature *v.* senescent	Young	K	J	—
	Mature	K	—	—
	Senescent	G, H, I, J, L	N	—

Consider first the indirect comparisons of different spindle leaves (Table 11). In two experiments (F and G) out of the three in which young leaves were compared indirectly with mature ones, there was a complete reversal from a clear preference for spindle when it was young to a clear preference for beet when the spindle was mature. In the third experiment (H) the inferiority of the mature to the young spindle leaves was less strikingly shown, for the mature leaves gained as many *wins* as the young beet standards; but their inferiority was nevertheless evident because the young spindle gained all the *wins* in the parallel test against young beet.

The superiority of senescent over mature spindle was evident, although in varying degree, in seven out of nine of the indirect comparisons of these leaves. In Exps. J and K the young beet leaves used as the standard of comparison were strongly preferred to both mature and senescent spindle leaves. Any possible superiority of senescent over mature spindle leaves was not detectable in Exp. J, and detectable in Exp. K only as a smaller proportion of *wins* for beet, and hence more *draws*, in the tests against senescent spindle leaves than in the parallel tests against mature ones.

The senescent spindle leaves were preferred to senescent beet leaves in five out of six experiments and, with the same exception (Exp. N), there was always some shift toward beet in the parallel tests of mature spindle against senescent beet. But the size of the shift ranged from a complete reversal from spindle preferred to beet preferred (Exp. G), to a mere reduction of spindle *wins* from six to five out of eight presentations (Exp. H).

TABLE 12. *Preferences among the leaves of one kind of plant: sugar beet. Classified results of outdoor experiments in which two types of sugar-beet leaf were compared indirectly, in parallel tests against one type of spindle leaf*

Beet leaves indirectly compared	Spindle leaf used as common standard for comparison	Experiments in which gross results showed		
		$Y > M$	$O = M$	$Y < M$
Young *v.* mature	Mature	F1, F, G1, H, J, K	I	—
	Senescent	J, K, O	—	—
		$O > M$	$O = M$	$O < M$
Mature *v.* senescent	Mature	F1, G, H, K	—	I, J
	Senescent	L, M, O	—	—

The results of the indirect comparisons of different beet leaves (Table 12) were even more variable in detail. Young beet leaves proved superior to mature ones in eight out of nine experiments. In Exp. I, even the mature beet leaves gained 100% *wins* in tests against the mature spindle standards, so that any possible superiority of the young beet leaves over the mature ones could not be detected. This exceptional behaviour of the aphids occurred when the weather was also exceptional, during the second half of July 1948; the only hot period in an unusually cold and wet year. The probable connexion between the weather and the changed behaviour of the aphids will be discussed in a later paper.

Senescent beet leaves proved superior to mature ones in seven out of nine comparisons. In Exps. I and J the opposite occurred. As far as Exp. I is concerned, the result might again have been connected with the hot dry weather, for the beets were severely wilted during the heat of the day and the outer, senescent leaves were more wilted than the mature ones. The results of Exp. J are fewer and may not be significant, but remain anomalous, for by that time the spell of fine weather had ended. In Table 13, the numerical results of the indirect comparisons of mature and senescent beet leaves are quoted both including and excluding Exps. I and J, to show that, apart from these experiments, the senescent beet leaves proved superior to mature ones.

TABLE 13. *Preferences among the leaves of one kind of plant. Combined numerical results of outdoor preference experiments in which two types of leaf on one plant were compared indirectly, in parallel tests against one type of leaf on the other plant.* Wins *for each plant summed from all experiments (Tables 9 and 10) involving each of the indirect comparisons indicated*

Plant	Leaf types indirectly compared	Total present-ations	Total *wins*	*Wins* (%) Spindle	Beet
Spindle	Young *v.*	27	12	100	0
	mature	27	18	17	83
	Mature *v.*	71	29	35	65
	senescent	79	39	69	31
Beet	Young *v.*	105	67	34	66
	mature	86	45	73	27
	Mature *v.*	81	43	72	28
	senescent	87	36	75	25
		Excluding Exps. I and J			
	Mature *v.*	67	31	94	6
	senescent	73	31	71	29

Feeding preferences between leaves of different plants

Given the existence of such preferences among leaves of different ages on the same plants, the main question was how far leaf age-differences could determine the aphids' preferences as between leaves of the two different host plants, spindle and beet. Experiments were therefore designed to show whether the aphids' preference could be directed at will toward one host or the other by choosing leaves of appropriate ages for caging together. For the first experiment, in the greenhouse, batches of leaves of two types, *YY* and *YM*, were selected for uniformity within the type from among the leaves on one pot spindle and a number of similar pot beets. As between the *YY* leaves of the two kinds, it will be seen from Table 14 that the spindle was preferred, although more slowly-growing. But the *YY* beet leaves were equal or just preferred to the *YM* spindle leaves, the beet growth-rate being here many times greater than that of the spindle. The spindle leaves regained their ascendancy

completely when both spindle and beet leaves were *YM* and so growing at less discrepant rates, although even here the beet was growing faster than the spindle.

TABLE 14. *Compound preference experiment (pot plants): shift of preference between spindle and beet according to the age of the leaves used (April 1948)*

Leaves compared									
Spindle		Beet							
Type	Growth rate (%)	Type	Growth rate (%)	No. cages	Total present-ations	*Wins* Spindle	*Wins* Beet	Simple majorities Spindle	Simple majorities Beet
YY	17	*YY*	30	9	9	5	1	7	2
YM	1	*YY*	26	9	9	3	4	2	7
YM	1	*YM*	9	6	6	6	0	6	0

Three of the outdoor experiments (Table 9, Exps. A, D 1 and E), done in the spring of 1948, when young spindle leaves were freely available, were designed with the same object of demonstrating shifts of preference with changes of relative growth-rate as between spindle and beet. In each experiment, two comparisons were made in parallel: α, fast-growing spindle leaves *versus* slow-growing beet leaves; and β, slower-growing spindle leaves *versus* faster-growing beet leaves. As between one experiment and another the results were, as usual, not altogether consistent. In Exp. A, when the spindle : beet growth-rate ratio was 4 : 21, *wins* were shared equally between spindle and beet, whereas in Exp. D 1 when the ratio was 4 : 24, and in Exp. E when it was 5 : 12, all the *wins* were gained by the spindle. Nevertheless within any one experiment the aphids' distributions differed, as between the α and β cages, as expected from the difference in relative growth rates. Here again the spindle was preferred to the beet not only when the spindle was growing faster (Exp. A, α) but also when it was growing more slowly than the beet (α, Exps. D 1 and E), and the beet was preferred to the spindle only when the discrepancy in growth-rates was very great (β, Exps. D 1 and E).

In practice, not only those three specially designed experiments but the whole series of outdoor experiments provide material for examining the effect of leaf age on the relative attractiveness of spindle and beet. The results listed in Tables 9 and 10 are therefore summarized in Table 15 and Fig. 4 from this point of view.

Since no two experiments were strictly alike the whole series is best presented (as in Tables 11 and 12) simply by classifying the experiments according to the gross result of each comparison. Thus only three classes of result are recognized here: spindle preferred, beet preferred and no preference. Whichever leaf gained a simple majority of *wins* is taken as preferred. To consolidate the data for Table 15 further (again as in Tables 11 and 12), leaves that were *YY* or *Y* are both classed as young; *YM* or initially *M* leaves are classed as mature; and initially *M*(*O*), *MO* or O_1 leaves are all classed as senescent.

It is apparent from Table 15 and Fig. 4 that young and senescent spindle leaves

TABLE 15. *Preferences as between spindle and beet leaves. Classified results of outdoor experiments*

Leaf types compared Spindle *v.* beet	Experiments with preferred leaves: Spindle	Experiments with preferred leaves: Beet	Experiments showing no preference
Young *v.* young	A, B1, C, D1, E, F, H	D	A, B
Young *v.* senescent	B, B1, C, G	—	—
Mature *v.* young	—	D1, E, F1, F, G1, I, J, K	H
Mature *v.* mature	F1, F, G, G1, H	I, K	J
Mature *v.* senescent	F1, H, I, J, L	G, K, N	—
Senescent *v.* young	O	G, J, K	—
Senescent *v.* mature	J, K, L, M, O	—	—
Senescent *v.* senescent	G, H, I, L, O	M, N	—

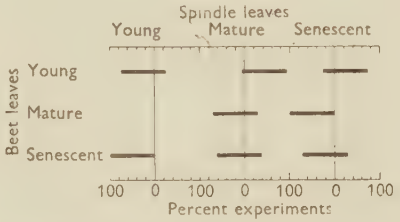


Fig. 4. Summarized results of outdoor preference experiments as between spindle and beet leaves. Data from Table 15. The result of each comparison between the various leaf types indicated along the top and left-hand margins, is shown by the extension of the horizontal block to the left (for spindle) and right (for beet) of the vertical lines, on a percentage scale as in Fig. 3. Thus for example young spindle leaves were preferred to senescent beet leaves in 100% of experiments.

were preferred to beet leaves more often than were mature spindle leaves. Young and senescent beet leaves were preferred to mature or senescent spindle leaves more often than were mature beet leaves. Thus the relative age of the leaves compared did apparently help to decide whether the spindle or the beet was preferred, along the lines expected if young and senescent leaves be superior to mature ones. At the same time, it is no less apparent that the results are only partially explicable on the simple principle that young and senescent leaves are superior to mature ones.

First, as already indicated by Tables 5, 6 and 7, it appeared that young leaves of either plant were superior to senescent leaves both of the same and of the other plant. As between the plants, there was one exception to this, Exp. O, when senescent spindle leaves were preferred to young beet leaves. But this experiment was done in November when the beet plants were in their resting winter condition with their young leaves growing very slowly, while the spindles were senescing rapidly. No

experiments involving young beet leaves were done in September or October when similar results might have been expected.

Secondly, the two plants did not behave as if they were one, the only differences present among their leaves being due to age. Spindle leaves of each age-group — young, mature and senescent—proved, on the whole, superior to their age-equivalents among the beet leaves. This is the more striking when it is remembered (Tables 9 and 10) that the young beet leaves were usually growing faster than the young spindle leaves; that the mature beet leaves were usually still growing a little as well as beginning to senesce, whereas the mature spindle leaves were doing neither; and that the senescent beet leaves were (up to Exp. K inclusive) senescing more rapidly than the senescent spindle leaves which remained in type *M*(*O*) for weeks together and often dehisced without becoming any paler. On all these counts the beet leaves should have had the advantage over their age-equivalents on the spindle, if growth and senescence alone were concerned in determining preferences. The conclusion that spindle leaves had some intrinsic superiority over beet leaves, is further supported by the fact that, while mature spindle leaves were preferred to senescent beet leaves in five experiments out of eight, mature beet leaves were never preferred to senescent spindle leaves.

Thirdly, there were gross inconsistencies between one experiment and another in the relative attractiveness of spindle and beet, some of which could hardly be put down to random variations or errors of technique. These may have been connected with changes in the turgor of the plants, to be discussed in a later paper.

Comparative fecundity among leaves of the same plant

Table 16 contains the results of seven experiments in which alatae were kept overnight in single-leaf cages, simultaneously on young beet leaves and on young and mature spindle leaves growing on pot plants in the greenhouse. Within each experiment the same pot plant provided both the young and mature spindle leaves, except in Exp. 1 where a second newly sprouting pot spindle provided the very young leaves. The figures of comparative reproduction rates shown in the last column of the Table were obtained by dividing the total number of young counted at the end of the experiment in all cages on leaves of a given type, by the total number of parents introduced into those cages the day before (approximately ten per cage). In every experiment, more young were produced per parent on the young spindle leaves than on the mature ones. And in Exp. 1 four times as many young were produced on the faster-growing as on the slower-growing young spindle leaves. Further greenhouse experiments, giving similar results, are given in Table 21 (p. 56).

The percentages of parents settled a few hours after introduction and surviving at the end of the experiments are included in Table 16, but these figures are of less value for comparative purposes than the figures of young produced per parent caged. The percentages are based on small numbers of insects; and accidental deaths, of which there were always some due to damage during transfer, introduced rather

large random variations in the percentage survival. The differences between the percentages surviving paralleled the differences in reproduction rate in four out of seven experiments in Table 16.

TABLE 16. *Comparative reproduction rates, percentages settled and percentages surviving in greenhouse experiments comparing young beet leaves with young and mature spindle leaves*

		Leaf			Parents		
Exp.	Plant	Type	Growth rate (%)	No. cages	Settled (%)	Survival (%)	Young per parent
1	Spindle	*YY*	23	3	100	93	4·1
	,,	*Y*	6	2	80	85	0·9
	,,	*M*	0	2	20	0	0·0
	Beet	*YY*	31	3	93	70	1·7
2	Spindle	*YY*	17	5	28	74	3·8
	,,	*M*	0	5	48	78	2·7
	Beet	*Y*	8	5	32	54	1·5
3	Spindle	*Y*	9	5	92	71	3·2
	,,	*M*	0	3	37	13	0·5
	Beet	*YY*	27	2	83	83	1·9
4	Spindle	*Y*	5	3	86	86	1·9
	,,	*M*	0	2	64	47	0·5
	Beet	*YY*	50	2	95	80	2·6
5	Spindle	*Y*	4	2	87	70	2·2
	,,	*M*	0	2	75	50	0·9
	Beet	*YY*	22	2	66	70	2·0
6	Spindle	*Y*	3	4	80	36	1·6
	,,	*M*	0	4	45	77	1·1
	Beet	*YM*	4	4	61	77	2·3
7	Spindle	*YM*	0·4	4	42	57	1·8
	,,	*M*	0	5	48	78	0·8
	Beet	*YM*	4	4	41	74	1·7

Differences in the proportion of aphids settled on isolated leaves, where the insects must feed or die, are plainly less valuable as a measure of preferences between leaves, than the results obtained in the two-leaf cages where the aphids were free to move from one leaf to another. Fig. 5 shows the changing proportions of live parents found settled in the cages in Exp. 5 of Table 16, from the time of introduction at 15.00 until 18.00 hr. that evening and again next morning from 06.00 hr. until the conclusion of the experiment when the young were counted. Considering first the combined curve for all six cages, an appreciable minority of the aphids were wandering rather than feeding during the few hours before dusk, but then they all settled on the leaves. A few began wandering again during the night but the great majority remained settled until after the morning sun fell on the cages. An hour and a half after that the majority were wandering and continued so until the experiment concluded.

This was the typical course of events in the single-leaf cage experiments as a whole (although early morning counts were not usually taken): a moderate amount of wandering in the afternoon after being caged, almost complete settling during the night, and active wandering again after sunrise the following morning. The production of young began in the afternoon but most of it occurred during the night. Practically none occurred during the following morning, and although some aphids were to be found with their mouth-parts inserted at any given moment during this period, the inserted aphids were found in new positions at successive readings, instead of in the same positions as they were found in the evening, so that the individuals did not appear to remain feeding for long. Deaths occurred mainly during the

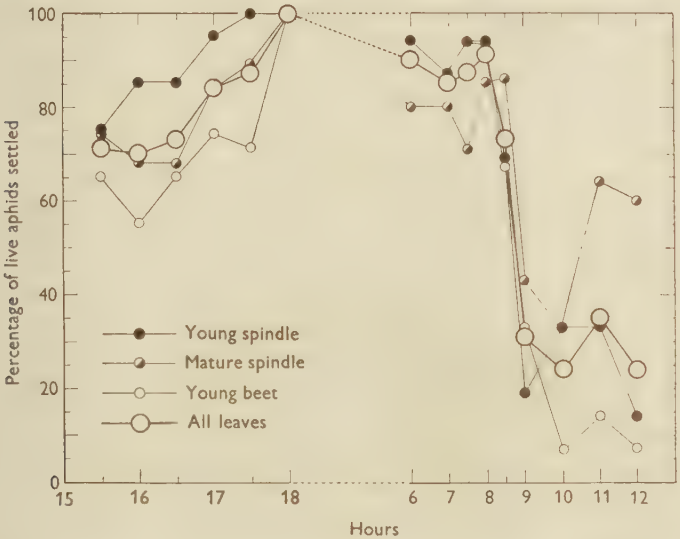


Fig. 5. Percentages of aphids settled on three types of leaf in single-leaf cages during the course of Exp. 5 of Table 16. The percentages were the same on the young spindle and young beet leaves at 6, 7 and 7½ hr.

morning when some of the aphids seemed to exhaust themselves in constant activity including attempts to fly.

Considering the separate curves for the three types of leaf (Fig. 5), it will be seen that the proportion of parents settled was higher on the young spindle leaves than on mature spindle leaves in the afternoon and early morning, which corresponds with the subsequent finding that more young aphids had been produced during that time on the young than on the mature spindle leaves. During the following morning, however, the situation reversed, and more aphids were found settled on the mature than on the young spindle leaves. This kind of reversal was observed on a number of occasions and may have been due to the increasing tendency of aphids to insert their

mouth-parts, if only for short periods, as they become starved (Ibbotson, in preparation). Thus the aphids which had been able to feed on preferred young leaves, may have become satiated and so free to wander again next morning, whereas those that had had access only to less acceptable leaves were still hungry and so probed repeatedly.

TABLE 17. *Summarized results of outdoor experiments on comparative reproduction rates on spindle and beet leaves of various ages (April–June 1948)*

			Leaf			Parents		Young per live parent per day
Exp.	Date	Plant	Type	Growth rate (%)	No. cages	Settled (%)	Survival (%)	
A	8–10 Apr.	Spindle	*Y*	7	5	99	92	2·4
		Beet	*Y*	13	5	87	90	0·8
		,,	*M*–?	3	5	79	80	0·8
		,,	O_1–?	2	5	84	78	1·1
A1	13–16 Apr.	Spindle	*Y*	3	3	46	56	2·2
		Beet	*Y*	13	5	72	78	1·9
		,,	*M*–?	4	4	63	37	1·2
		,,	O_1–?	0·4	5	68	53	1·5
B	20–22 Apr.	Spindle	*YY*	11	5	42	28	2·9
		Beet	*YY*	29	5	54	60	2·6
		,,	*M*–?	3	5	27	39	1·5
		,,	O_1–?	1	5	22	44	2·7
C	26–28 Apr.	Spindle	*Y*	3	3	40	37	3·9
		,,	*YM*	0·4	2	44	50	1·9
		Beet	*YY*	34	5	65	60	2·8
		,,	*M*–?	2	5	45	56	2·0
		,,	O_1–?	0·3	5	54	44	2·7
D	4–6 May	Spindle	*Y*	2	5	45	58	2·4
		Beet	*YY*	26	5	62	52	1·6
E	19–21 May	Spindle	*YY*	17	2	33	85	2·7
		,,	*YM*	0·4	2	46	33	1·2
		Beet	*Y*	17	5	60	46	2·3
F	22–25 June	Spindle	*Y*	4	3	24	50	2·1
		,,	*YM*	2	2	18	30	0·8
		,,	*M*(*O*)	0	5	51	56	1·9
		Beet	*YM*	7	5	36	48	1·6
		,,	*M*–*M*(*O*)	0	3	41	37	1·8
		,,	*M*–*MO*	0	2	57	45	2·8
G	29 June–1 July	Spindle	*Y*	4	4	67	81	1·1
		,,	*M*	0	4	57	93	0·6
		,,	*MO*	0	4	50	100	1·2
		Beet	*YM*	5	4	70	87	1·2
		,,	*M*–*M*(*O*)	0·3	4	71	84	0·6
		,,	O_1–O_2	0	2	75	80	1·7
		,,	O_2–O_4	0	2	45	95	0·7

For that reason the figures of percentages of parents settled given in Table 16 are averages of several successive readings during the afternoon, and do not refer to the behaviour of the aphids when they become active again next morning. It will be

TABLE 18. *Summarized results of outdoor experiments on comparative reproduction rates on spindle and beet leaves of various ages (July–November 1948)*

Exp.	Date	Plant	Leaf: Type	Leaf: Growth rate (%)	No. cages	Parents: Settled (%)	Parents: Survival (%)	Young per live parent per day
H	6–9 July	Spindle	*Y*	4	4	62	69	1·7
		,,	*M*	0	4	57	93	2·7
		,,	*M(O)*	0	4	70	73	2·4
		Beet	*Y*	12	4	62	68	1·7
		,,	*M–M(O)*	0·5	4	55	77	1·6
		,,	*M(O)–MO*	0	4	53	77	1·9
H1	14–17 July	Spindle	*M*	0	3	50	47	2·4
		,,	O_1–O_2 (artif.)	0	3	77	60	3·1
I	27–30 July	Spindle	*Y*	2	4	64	23	3·3
		,,	*M*	0·6	4	58	15	2·4
		,,	*M(O)*	0	4	0	0	1·8
		Beet	*YY*	59	4	39	33	3·9
		,,	*M*	4	4	28	27	2·6
		,,	*M(O)*–O_2	1	4	0	0	0·0
J	4–7 Aug.	Spindle	*M*	0	4	49	47	2·3
		,,	*M(O)*	0	4	75	65	2·6
		Beet	*Y*	16	3	61	47	2·3
		,,	*M*	1	4	68	57	2·9
		,,	*MO*–O_1	0	3	64	48	1·9
K	10–13 Aug.	Spindle	*M*	0	3	53	54	1·8
		,,	*M(O)*	0	5	72	69	3·4
		Beet	*YY*	52	4	78	52	2·6
		,,	*M–M(O)*	0·2	4	83	54	1·4
		,,	*MO*–O_1	0	3	78	48	1·9
L	28 Sept.–1 Oct.	Spindle	*M–M(O)*	0	3	35	45	2·1
		,,	*MO*	0	3	26	73	2·5
		Beet	*YY*	39	3	30	74	0·7
		,,	*M*	3	3	27	73	1·1
		,,	*MO*–O_1	0·3	2	25	69	1·0
		,,	O_3–O_4	0	3	19	83	0·7
M	12–16 Oct.	Spindle	*MO*	0	3	63	83	1·1
		Beet	*Y*	11	3	59	57	0·5
		,,	*M–M(O)*	1	3	67	72	0·8
		,,	*MO*–O_1	0	3	56	89	1·0
		,,	O_3–O_4	0	3	50	43	0·4
N	19–23 Oct.	Spindle	*M–M(O)*	0	5	29	81	0·7
		,,	O_1–O_2	0	2	38	90	2·3
		,,	O_2–O_4	0	2	26	59	0·5
		Beet	*M(O)*	0	5	34	79	0·6
		,, (bolter)	*M(O)*–O_2	0	3	32	67	0·6
O	3–5 Nov.	Spindle	*MO*–?	0	6	40	58	3·0
		Beet	*Y*	?	10	61	74	1·9
		,,	*M*	?	8	53	64	1·8
		,,	*MO*	?	6	55	77	2·0

seen that there were more parents settled on the young than on the mature spindle leaves, corresponding with the production of more young aphids on the young leaves in five out of the seven experiments in Table 16. The opposite was true in Exps. 2 and 7, although the reproduction rates were not greatly different as between the two-leaf types in Exp. 2 and the settling rates were not greatly different in Exp. 7. The relation between settling and reproduction will be considered further in a later section (p. 54).

The outdoor reproduction experiments are listed in Tables 17 and 18. They are lettered to correspond with the lettering of the preference experiments done concurrently. Reproduction experiments which were done alone, without a concurrent preference experiment, have the figure 1 after the letter. Experiment H1 was designed to test whether spindle leaves further advanced in senescence than type *MO*, which are available in masses in the autumn but are rare or absent (except as a few scale leaves due to dehisce) in the summer, were superior to the *M* type leaves which comprised the great majority in the summer. The senescent spindle leaves used for this purpose were on a pot plant which had been forced to sprout and mature in the greenhouse in winter–spring and then kept outdoors, and had therefore begun to senesce several months before the spindles growing naturally outdoors did so. It was senescing rapidly, with some leaves already falling, when it was set alongside a naturally-growing spindle for Exp. H1. The soil around the pot plant was certainly not moister and was probably considerably drier than that around the naturally rooted plant. Exp. O was carried out on outdoor-grown plants, but in the greenhouse, as explained with regard to preference Exp. O (p. 37).

Tables 17 and 18 include the percentages of parents settled during, and surviving at the end of the experiments, but these figures are again of less comparative value than the reproduction figures, for the reasons already given. The figures of percentages settled are based on counts done only once each day, in the early afternoon, which appeared to be the time when contrasts in the proportions of alatae wandering on different leaf types were usually at a maximum. In the morning there were often too many wandering for differences to show, and in the evening the aphids were nearly all settled on all types of leaves.

The aphids were left caged on the leaves usually for 3 and sometimes for 4 days, depending on the rate of young-production. The number of surviving parents was counted at dusk every day and also on the morning of the last day when the cages were cleared. In order to simplify calculation of the proportion of parents settled, while taking account of the declining total of live parents in the cages, the number recorded as settled in all the cages on leaves of a given type was summed for the successive days of an experiment, and the sum expressed as a percentage of the sum of the parents alive in all those cages at the start of each successive day. For example, given thirty aphids (three cages) introduced originally, if twenty-four were found alive the first night and eighteen the second, and if eighteen were found settled the first afternoon, fifteen the second and nine the third, then the percentage settled was estimated as: $100 \times (18+15+9)/(30+24+18) = 58\%$.

Reproduction rates were similarly calculated. In the same example, with a total of twenty-four aphids alive the first night, eighteen the second and twelve the third, if a total of 150 young were counted in the three cages at the end of the experiment on the fourth morning, then the reproduction rate was estimated as:

$$150/(24+18+12)=2{\cdot}8 \text{ young/live parent/day.}$$

TABLE 19. *Comparative reproduction rates, etc. among leaves of one kind of plant. Outdoor experiments classified according to whether more or less young were produced, per live parent per day, on young and senescent leaves than on mature ones; and re-classified in the same terms for percentage settling and survival of parents*

		Experiments giving indicated result		
Plant	Result	Settling (%)	Survival (%)	Reproduction
Spindle	$Y>M$	F, G, H, I	E, F, I	C, E, F, G, I
	$Y<M$	C, E	C, G, H	H
	$O>M$	F, G, H, H1, J, K, N	F, G, H1, J, K, L, N	F, G, H1, J, K, L, N
	$O<M$	I, L	H, I	H, I
Beet	$Y>M$	A, A1, B, C, H, I, L, O	A, A1, B, C, F, G, I, O	A1, B, C, G, H, I, K, L, O
	$Y=M$	G	H, K, L	A
	$Y<M$	J, K, M	J, M	F, J, M
	$O>M$	A, A1, C, F, G, O	A, A1, B, F, M, O	A, A1, B, C, F, G, H, K, L, M, O
	$O=M$	—	G, H, L	—
	$O<M$	B, H, I, J, K, L, M	C, I, J, K	I, J

The reproduction experiments, with only one type of leaf in each cage, provided a direct comparison between leaves of the same plant and between leaves of different plants at the same time. The results are analysed in Table 19 for comparison of leaves of the same plant. The experiments are simply classified, as were the preference experiments in Tables 11, 12 and 15, in terms of their 'gross results', i.e. according to whether:

More young were produced per live parent per day on young or senescent leaves than on mature leaves:

$$Y>M \quad \text{or} \quad O>M;$$

Or, More young were produced on mature than on young or senescent leaves:

$$Y<M \quad \text{or} \quad O<M,$$

Or, There was no difference between the reproduction rates on these leaves:

$$Y=M \quad \text{or} \quad O=M.$$

The last column of Table 19, and Fig. 6, show that, in most cases, young and senescent leaves of both spindle and beet permitted more young to be produced by the average live parent, than did mature leaves of the same plant. Experiments in

which reproduction was more rapid on mature than on young or senescent leaves were relatively few. Experiments in which the percentages of parents settled and surviving were higher on mature than on young or senescent leaves, were more numerous, but again in a minority, except for the results on settling as between mature and senescent beet leaves (seven experiments giving $O < M$ against six giving $O > M$), and on survival as between young and mature spindle leaves (three experiments giving $Y > M$ and three giving $Y < M$).

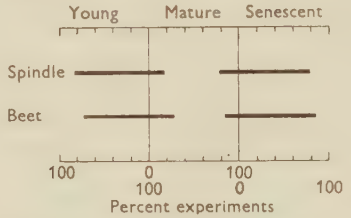


Fig. 6. Summarized results of outdoor comparative reproduction experiments among leaves of the same kind of plant. Data from Table 19, presented as in Fig. 3.

Exp. 1, done in the 'heat-wave' at the end of July, stands out from the rest, as did preference Exp. 1 done at the same time. The results were then very uneven among cages on leaves of the same type, and survival was generally low. The aphids evidently found the senescent spindle and beet leaves highly unsuitable, for the parents were extremely active in the cages and all dead on the senescent beet and 70% dead on the senescent spindle at the close of the first afternoon.

Comparative fecundity among leaves of different plants

In the greenhouse experiments on young and mature spindle and young beet leaves (Table 16), relative reproduction rates as between spindle and beet followed much the same pattern as found in the preference experiments on such leaves. More young were produced per parent on young spindle than on young beet, both when the spindle leaves were growing faster than the beet leaves (Exp. 2), and even when the spindle leaves were growing more slowly than the beet (Exps. 3, 5 and 7). The reproduction rate was higher on young beet than on young spindle leaves when the beet leaves were growing very much faster than the spindle leaves (Exps. 1 and 4), and higher on the young beet than on the mature spindle leaves in every experiment except no. 2. There were other inconsistencies, such as the lower reproduction rate on young spindle than on young beet when the spindle : beet growth-rate ratio was 2 : 4 in Exp. 6, as contrasted with the higher or nearly equal reproduction rates on young spindle when the growth-rate ratio was more favourable to beet (4 : 22 in Exp. 5 and 0·4 : 4 in Exp. 7).

The outdoor experiments listed in Tables 17 and 18 are analysed for comparison of leaves of the two different kinds in Table 20 and Fig. 7, by classifying them

TABLE 20. *Comparative reproduction rates, etc. as between spindle and beet leaves. Outdoor experiments classified according to whether more or less young were produced, per live parent per day, on spindle than on beet leaves of the various types; and re-classified in the same terms for percentage settling and survival of parents*

Leaf types compared		Experiments giving indicated result		
Spindle *v.* Beet	Result	Settling (%)	Survival (%)	Reproduction
Y v. *Y*	Sp. > Bt.	A, I	A, D, E, F, H	A, A1, B, C, D, E, F
	Sp. = Bt.	H	—	H
	Sp. < Bt.	A1, B, C, D, E, F, G	A1, B, C, G, I	G, I
Y v. *M*	Sp. > Bt.	A, B, H, I	A, A1, F	A, A1, B, C, F, G, H, I
	Sp. < Bt.	A1, C, F, G	B, C, G, H, I	—
Y v. *O*	Sp. > Bt.	A, B, H, I	A, A1, F, G, I	A, A1, B, C, I
	Sp. < Bt.	A1, C, F, G	B, C, H	F, G, H
M v. *Y*	Sp. > Bt.	I, L	G, H, K	H, L
	Sp. = Bt.	—	J	J
	Sp. < Bt.	C, E, F, G, H, J, K	C, E, F, I, L	C, E, F, G, I, K
M v. *M*	Sp. > Bt.	H, I, L	G, H	H, K, L
	Sp. = Bt.	C	K	G
	Sp. < Bt.	F, G, J, K	C, F, I, J, L	C, F, I, J
M v. *O*	Sp. > Bt.	I, H, L	C, G, H, I, K, N	H, I, J, L, N
	Sp. < Bt.	C, F, G, J, K, N	F, J, L	C, F, G, K
O v. *Y*	Sp. > Bt.	F, H, J, M	F, G, H, J, K, M	H, J, K, L, M, O
	Sp. = Bt.	—	—	G
	Sp. < Bt.	G, I, K, L, O	I, L, O	F, I
O v. *M*	Sp. > Bt.	F, H, J	F, G, J, K, M	G, H, K, L, M, O
	Sp. = Bt.	—	L	F
	Sp. < Bt.	G, I, K, L, M, O	H, I, O	I, J
O v. *O*	Sp. > Bt.	H, J, L, M, N, O	F, G, J, K, L, N	H, I, J, K, L, M, N, O
	Sp. = Bt.	I	I	—
	Sp. < Bt.	F, G, K	H, M, O	G

according to the gross results, as was done for comparison of leaves on the same plant in Table 19. The relative age of the leaves did, apparently, help to decide, along the lines expected if young and senescent leaves be superior to mature ones, whether the reproduction rate was higher on spindle or beet, in accord with the results of the spindle-beet preference experiments (Table 15). Thus, the reproduction rate was higher on young and senescent spindle leaves than on beet leaves of all ages, relatively more often than it was higher on mature spindle leaves than on beet leaves of all ages. And the reproduction rate was higher on young beet than on young spindle leaves, relatively more often than it was higher on mature beet than on young spindle leaves. Otherwise, however, the results are not explicable on the simple hypothesis that young and senescent leaves are superior to mature ones.

In the preference experiments, it will be recalled (p. 44), young leaves appeared to be superior to senescent leaves on the same or on the other kind of plant. This tendency was less consistently shown in the reproduction experiments and in some cases the reverse was true. On the other hand, the intrinsic superiority of spindle

over beet which was evident in the preference experiments, was paralleled in the reproduction experiments, although the parallel was not complete in detail. Thus the reproduction rates on young and senescent spindle leaves were usually higher than on their age-equivalents among the beet leaves, although among mature leaves, spindle and beet proved superior to each other about equally often. The intrinsic superiority of spindle leaves was further demonstrated by the following findings. While the reproduction rate on mature spindle leaves was higher than on young beet leaves in two out of eight experiments, the reproduction rate on mature beet leaves was never higher than on young spindle leaves. While the rate was higher on mature spindle leaves than on senescent beet leaves in five out of nine experiments, the rate on mature beet leaves was higher than on senescent spindle leaves in only two out of eight experiments. And, while the reproduction rate was higher on young spindle than on senescent beet leaves in five out of eight experiments, it was higher on young beet than on senescent spindle leaves in only two out of eight experiments.

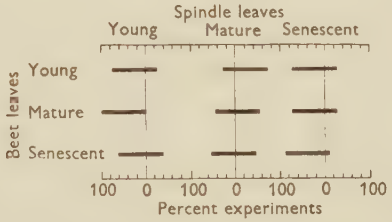


Fig. 7. Summarized results of outdoor comparative reproduction experiments as between spindle and beet leaves. Data from Table 20, presented as in Fig. 4.

It will be seen that the results on comparative reproduction rates as *between* the two different kinds of plant were altogether more variable and therefore more evenly balanced, than the results on comparative reproduction rates *within* each kind of plant. Also, the correspondence between the comparative results for settling and survival on the one hand, and reproduction rate on the other hand, in the same experiments, was poorer as between the two plants than within each one. And finally, the comparative reproduction results as between plants were more variable than the results of the preference experiments both within and between plants.

Fecundity as a variable independent of settling differences

When the average reproduction rate of aphids caged on one type of leaf was different from that on another, the difference might have been due merely to a behavioural difference, that is, to the aphids spending more time feeding on one leaf than another. Alternatively it might have been due to a true fecundity difference attributable to differences in the quantity and quality of the food obtained by the aphids while actually feeding. Having been done under semi-natural conditions the present

experiments were not well adapted for distinguishing these two effects. Counts of the numbers of parents settled, which would have to be taken into account in calculating the reproduction rate of *feeding* aphids, were taken mainly in the afternoon, whereas the bulk of the young were produced during the night. Thus the relation between the percentages of aphids settled on two different leaf types in the afternoon was not a safe guide to the relation between them at night.

For example, in Fig. 5 the percentage of parents settled was higher on beet leaves than on the spindle leaves during the night, which corresponds with the subsequent finding that more young had been produced on the beet. But the afternoon counts of settled parents did not entirely correspond with the final reproduction figures, for there was a higher percentage of parents settled on mature spindle than on the young beet leaves. Hence the large minority of comparisons of different leaf types in which there was a similar lack of correspondence between the afternoon counts of settled parents and the final reproduction rates, does not necessarily mean that reproduction rates were independent, even to some extent, of settling rates.

The only recorded data on settling at night are those for the early morning of Exp. 5 (Fig. 5). Taking the numbers of parents settled at 06.00 hr. and dividing them into the numbers of young finally counted in the cages at 12.00 hr., in order to obtain an estimate of the relative reproduction rates of *feeding* aphids, gives values of 2·8 young per parent on the young spindle, 1·5 on the mature spindle and 2·7 on the young beet leaves. In this instance it appears that the aphids were truly more fecund when feeding on young spindle and young beet leaves than when feeding on mature spindle leaves, quite apart from their greater readiness to settle and feed on the young leaves. But the reproduction rates are not significantly different in this one example. More experiments are required for direct proof of the independent existence of fecundity differences between aphids feeding on different leaves.

Meanwhile there is some indirect evidence on this point. Table 21 contains the results of greenhouse, single-leaf cage experiments not included in Table 16 because the two types of spindle leaf were not on the same plants and growth rates were not measured: unimportant matters for the present purpose. During Exps. 6 and 7 of Table 21, an unshielded 500 W. light bulb was suspended over the plants and caused the aphids in the cages on the apical, young leaves, nearest the light, to wander about more than they did when the light was off. This brought the percentages of aphids settled on the youngest leaves, throughout the experiments, down toward or even below the percentages settled on the older leaves.

The succession of counts taken during the afternoon after loading, in all the greenhouse experiments (Tables 16 and 21), gave a good measure of comparative settling in the single-leaf cages at that time. During the night, when most of the young were produced, no counts were taken, but it is known that most of the adults were settled and it is most unlikely that the proportions of parents settled on different leaf-types diverged significantly more during the night than during the afternoon. There are numerous cases in Tables 16 and 21 where the ratio between the reproduction rates

on two leaf types used in the same experiment was (to make the test severe) two or more times as great as the ratio between the percentages of parents settled in the afternoon. There are even a few cases where the reproduction rate on one leaf-type was two or more times greater than that on another, although there were actually fewer parents settled in the afternoon on the former than on the latter leaf.

TABLE 21. *Comparative reproduction rates and percentages of adults settled in supplementary greenhouse experiments*

Exp.	Plant	Leaf type	No. cages	Parents settled (%)	Young per parent
1	Spindle	*Y*	6	80	2·9
	,,	*M*	6	74	0·9
2	Spindle	*Y*	5	52	3·0
	,,	*M*	6	39	0·5
3	Spindle	*YY*	6	53	3·6
	,,	*M*	6	51	1·4
4	Spindle	*YY*	3	63	1·9
	,,	*M*	3	43	0·5
5	Spindle	*M*	6	42	0·8
	,,	O_2	5	22	1·0
	Beet	*YY*	4	18	1·7
6	Spindle	*Y*	5	48	4·2
	,,	*M*	4	33	1·8
	,,	O_1	5	35	2·0
7	Spindle	*YY*	6	63	4·4
	,,	*YM*	6	76	3·6
	,,	*M*	6	38	2·4
8	Spindle	*M*	3	21	0·6
	Beet	*Y*	3	12	1·8

Thus there were many cases where the difference in reproduction rates on different leaves was extremely unlikely to be due merely to the parents spending more time feeding on the leaves where more young were born, the reproduction rate of feeding aphids being the same on all leaves. In these cases the reproduction rate of feeding aphids was almost certainly higher on the leaves where more young were born. If so, it may be assumed that differences of fecundity, as well as of behaviour, contributed to the differences in average reproduction rate in other cases also.

DISCUSSION

Behaviour and fecundity

The relative susceptibility of different leaves to infestation by aphids was recorded both in terms of settling preferences and of average reproduction rates. The two-leaf cages provided unambiguous evidence that *Aphis fabae* exercises active behavioural preferences as between different leaves. The single-leaf cages showed that the average reproduction rate of the aphids varied when they were caged on different leaves, but

were more ambiguous, in that the reproduction rate depended not only on the fecundity of the aphids while they were feeding, but also on the amount of time they spent feeding rather than wandering round the cage. However, there was good if indirect evidence from some of the greenhouse experiments that the higher average reproduction rate of aphids caged on certain leaves as compared with others, could not have been due merely to the aphids spending more time feeding on those leaves, and must have been due partly to their being more fecund while feeding.

It does not necessarily follow that the sap of leaves on which fecundity was greatest, was the most nutritious. Although it can hardly be doubted that the sap of different leaves does vary in nutritive value, the rate of feeding was not recorded, so that it was impossible to distinguish between the effects, on fecundity, of nutrition in the strict sense—the *quality* of the food ingested, and the effects of behaviour—the amount of time spent feeding and hence the *quantity* of food ingested. The best that can be done is to distinguish the effects of variations in overt behaviour—the amount of time spent with the mouth-parts inserted in the leaf—from the combined effects of variations in the rate of uptake of sap and in the nutritive value of the sap taken up. The two latter, indistinguishable effects will be referred to together as the 'nutrition' effects in contra-distinction to the overt behavioural preferences.

The main finding was that, although separate behavioural and 'nutritional' effects exist, the two methods of measuring the effects on the aphids of variations between leaves, gave a series of results which fell into the same general pattern (cf. Fig. 3 with Fig. 6 and Fig. 4 with Fig. 7). In terms of preferences and in terms of reproduction rates, young and senescent leaves were generally superior to mature leaves, and spindle leaves generally superior to beet. The results of the individual experiments confirm this parallelism between the two kinds of effect. The same pairs of different leaf-types were compared simultaneously in single- and two-leaf cages outdoors, on a total of fifty-eight occasions (Tables 11, 12, 15, 16 and 20). Out of this total, the leaves preferred in two-leaf cages gave higher reproduction rates in the single-leaf cages in forty-four or 77% of the comparisons. Thus leaves that showed themselves superior in the one respect usually did so also in the other.

This conclusion must be qualified. Among the total of sixty-one single-leaf-cage comparisons between leaves of different ages on the *same* kind of plant (Tables 16, 19 and 21), there were fourteen cases or 23%, in which the comparative settling and comparative reproduction results contradicted one another: that is, when higher settling rates occurred on the leaves giving lower reproduction rates in the same cages. Whereas among the total of ninety-eight comparisons between leaves on the two *different* kinds of plant (Tables 16, 20 and 21) there were thirty-eight cases or 39%, in which the settling and reproduction results were contradictory. The difference between these two percentages is significant at about 3·5%, using the method of angular transformation and Table XII of Fisher & Yates (1943).

Among the outdoor experiments in which the same pairs of leaf-types were compared in single-leaf and in two-leaf cages simultaneously, 14% or three of the

twenty-one comparisons *within* one kind of plant gave results in the preference cages which contradicted the reproduction results in the single-leaf cages; whereas 27% or ten of the thirty-seven comparisons *between* the two kinds of plant did so. This difference is not in itself significant statistically owing to the rather small total number of comparisons involved. But the difference is based on a separate and better set of data on comparative settling, and is in the same direction as before. The spindle leaf was the one preferred for settling, but giving the lower reproduction rate, in seven of the ten contradictory cases. Thus fecundity and readiness to settle varied together, more often among leaves of the same plant than between leaves of the two different plants, spindle and beet.

The dual discrimination theory of host selection

One kind of discrimination clearly shown by the aphids was between leaves of different ages, young and senescent leaves being, on the whole, preferred to mature ones. The experiments thus confirmed the conclusions drawn from the study of free infestations on whole plants (Kennedy *et al.* 1950; Ibbotson & Kennedy, 1950). The experiments suggested that the aphids exercise this kind of discrimination not only among the leaves of the same kind of plant but also, if less clearly, as between leaves of different plants. In other words leaves on plants of different species (in this case, of different Orders: spindle tree—Sapindales, and beet—Centrospermae) seem to present certain differences connected with their physiological development which are, for the aphids, of the same order and kind as the differences presented by leaves on the same plant.

The aphids' preferences were not only more consistently related to leaf age, among the leaves of one kind of plant than between the two different plants. Their preferences were also more consistently related to their fecundity differences, among the leaves of one kind of plant than between the two different plants. Since the fecundity differences were partly attributable to varying nutrition, it is proposed to call the kind of behavioural discrimination which is related to leaf age, a *nutrient* discrimination.

The choice of the term nutrient is a mere descriptive convenience. It does not imply that stimuli to which the aphids respond behaviourally, necessarily come from the same substances or properties as those most important to them nutritionally. Nor does it imply that the second kind of discrimination, that between *kinds* of plants and to be considered next, is associated with properties or substances not at all important in the nutrition of a given aphis species. It implies only that the properties or substances to which the aphids respond behaviourally in selecting among leaves of different ages, are more closely associated with their nutritional requirements than the properties to which the aphids respond when selecting between the leaves of two kinds of plant.

The term in no way excludes reactions other than gustatory ones, before or after insertion of the mouth-parts, from some part in the exercise of this kind of pre-

ference. The reactions involved may be partly olfactory, tactile, etc. (see discussion in Kennedy & Booth, 1950).

The existence of a second kind of discrimination was strongly suggested by a variety of inter-connected facts. Both the preference results and the reproduction results, considered independently, followed the rule that young and senescent leaves are superior to mature ones, less closely when spindle and beet leaves were being compared than when leaves on either plant alone were being compared. The greater inconsistency was not random, for the spindle leaves proved superior, on the whole, to beet leaves, to a considerable extent regardless of age. Thus there seemed to be a preference for one *kind* of plant, spindle, alongside the preference for particular *ages* of leaf on either plant. The postulated second type of discrimination we may call a *flavour* discrimination in contra-distinction to the so-called nutrient discrimination, and again without assuming that the so-called flavour discrimination depends only on gustatory reactions. On the available evidence, the flavour discrimination is just as likely to be negative as positive i.e. it may be a tolerance of, or it may be a preference for, certain 'flavours'.

It seems very difficult to account for the aphids' preferences without assuming at least these two main kinds of discrimination on their part. A simpler hypothesis would be that the aphids react to the same kind of substances or properties in the two kinds of plant, but find them generally nearer to some optimum condition in the spindle than in the beet. The immediate difficulty here is that readiness to settle and fecundity varied generally together, in the same direction as between two leaves, so that fecundity can be regarded as a rough indicator of the plant-properties discriminated behaviourally; and yet they varied together significantly less often in comparisons between plants than within them (p. 57). If the aphids' discrimination between leaves on different plants depended only on reactions to the same properties as those determining their discrimination between leaves of different ages, then it is difficult to understand why preferences should be associated with fecundity differences, more closely within than between plants.

The discrepancy is understandable if two kinds of discrimination are recognized, one between properties associated with the age of a leaf and one between properties associated with the kind of leaf. Fecundity differences depend not only on behavioural discrimination but also on nutrition, whereas preferences depend only on behavioural discrimination. If therefore one kind of discrimination, that concerned with age of leaf, is between properties more closely associated with nutritional properties than the other, that concerned with kind of leaf, then preferences should mirror fecundity differences more often when leaves differing only in *age* are compared, than when leaves differing also in *kind* are compared.

A more general difficulty with any hypothesis assuming that discrimination between leaves is by reactions to one class of properties only, is the extreme diversity of host relations among aphids which are otherwise a remarkably uniform group. This combination of uniformity with diversity suggests not one but at least two kinds of leaf-

discrimination reaction: one subserving the elaborate ecological differentiation of aphids, and the other their probably more uniform nutritional requirements. More colloquially expressed, one might expect aphids to have evolved some tastes for things that are good for them in an immediate nutritional sense, and other tastes for things that are less or not at all good for them in that sense but are valuable as instruments of ecological specialization.

In the particular instance of *Aphis fabae* the dual discrimination theory assumes that the specific flavour of spindle is preferred to (or alternatively, better tolerated than) that of sugar beet, and that both these flavours are preferred to the flavours of the many kinds of plant not colonized by *A. fabae*. At the same time it is suggested that *A. fabae* reacts not only physiologically (nutritionally, with repercussions on fecundity), but also behaviourally to certain nutrients, or properties or substances associated with the nutrients. These are assumed to occur in spindle, beet and all the other hosts of the aphid, as well as in other plants, but to vary in amount with the age of the leaves. And it is assumed that the aphids must receive suitable sensory stimuli of both kinds, flavour and nutrient, before they will stay to colonize leaves, so that a plant of the right species may be totally unacceptable if it is in the wrong physiological condition.

The discrimination of food objects by means of nutritionally unimportant 'token' substances, is well established among insects. Dethier (1947) reviews the evidence as far as olfactory discrimination is concerned, and points out that since physical stimuli such as light act as token stimuli, it is not strange that some chemical stimuli are also non-nutritious tokens. Dethier goes much further, however, and says (p. 71): 'It is now well-established that plants are recognized by the feeding stages of insects through the agency of odours.' He admits (ibid. p. 252) an important role for gustatory discrimination only in subterranean root-feeders (as wireworms: Thorpe, Crombie, Hill & Darrah, 1947), or in selection between different plant *tissues* during probing by aphids and leaf-hoppers (Fife & Frampton, 1936). In selection between whole leaves and whole plants, he discounts any gustatory or other discrimination connected with nutritional requirements:

> Since the nutritive value of various angiospermous leaves is not markedly dissimilar, as evidenced by the fact that a given monophagous larva may by various experimental devices be fed on a variety of plants, factors governing choice may be largely divorced from nutritional requirements. (Dethier, 1947, p. 39.)

A similar conclusion was drawn by Chin (1950) from a physiological analysis of the host relations of *Leptinotarsa decemlineata* Say, in which gustatory discrimination was shown to play, in fact, an important part:

> An attractive smell and taste, absence of poisons, and high nutritive contents by no means always go hand in hand together; they seem to combine just by chance and into sixteen possibilities. Maybe all these possibilities exist (p. 75).

It is clear from Chin's work that the four relevant properties of host plants do occur in varying combinations in different members of the *Solanaceae*. But to suggest that these properties may vary quite independently, their combinations being purely

random, implies that the insect always reacts to token properties which merely happen to be associated with satisfactory nutrition; it never reacts to properties having a necessary connexion with its nutrition. It seems most improbable that behaviour and nutrition could be so divorced, and they are not so in *Aphis fabae*. While the aphids do react discriminately to stimuli peculiar to the kind of plant and apparently of little importance nutritionally, they also react to stimuli peculiar to the age of leaf and connected with nutrition.

The dual discrimination theory should not be confused with the familiar fact that insects generally gain their appropriate food, oviposition site, etc., by a process which may be divided roughly into two stages: finding and selection (Salt, 1938 *et ante;* Thorpe *et al.* 1947; Chin, 1950). In that kind of behaviour pattern the two successive kinds of stimulus provided by the appropriate objects in nature, are as a rule both positive. The dual discrimination theory also postulates two kinds of stimulus but here we are concerned, in effect, with only one essential reaction: staying to feed or leaving. The natural objects in question—leaves—furnish both kinds of stimulus, but here we do not postulate that the two kinds usually work together.

To the human tongue, the specific flavour of spindle and sugar-beet leaves seems strongest when they are mature; very young and very old leaves are relatively insipid. This may hold for any animal which discriminates between plant kinds. Doubtless the specific flavour of any plant could, if concentrated, become eventually too strong for an aphid, as for man. And the concentration of flavour in mature leaves may sometimes be above the level preferred by the aphids even in appropriate kinds of host-plant. But it is most unlikely that the specific flavour and the nutrient properties of sap usually occur at their optimum level, for the aphids, in the same leaves. Rather, it appears that the supplies of flavour and nutrient change in opposite directions through the life of a leaf, with flavour increasing and nutrient decreasing on the way to maturity, and flavour decreasing and nutrient increasing again (temporarily) after it. This would be consistent with the finding (Kennedy, Ibbotson & Booth, 1950) that *A. fabae* colonized the middle-aged leaves of sugar beets, relatively to the young and senescent ones, to a greater extent than did *Myzus persicae*. *M. persicae*, on the other hand, colonized the very young and very old leaves to a relatively greater extent than *Aphis fabae*. This difference between the two aphid species was even more pronounced on *Euonymus* leaves. On general grounds, sugar beet would be regarded as more specifically *Aphis fabae*'s host than *Myzus persicae*'s; and *Euonymus*, again, even more so. This combination of facts is what we should expect if the process of adaptation of an aphid to a given kind of plant were a matter of the aphid acquiring a preference for the specific flavour of the plant, and if that flavour were strongest in the mature leaves. An aphid which had become closely adapted to a given plant would then find the youngest and oldest leaves too flavourless, and would colonize leaves nearer to maturity for preference, even if they were less nutritious. A less specifically adapted aphid would colonize leaves further from maturity.

As a rider on the dual discrimination theory, therefore, it is assumed that nutrients usually occur at their preferred level in young and senescent leaves containing less than the preferred concentration of specific flavour, and that the specific flavour usually occurs at its preferred concentration in more middle-aged leaves containing less than the preferred level of nutrients. In that case, the aphids would often encounter leaves which were satisfactory in one respect but not in the other. The leaves actually colonized would be those offering the best compromise between the aphids' two fundamental, but in nature essentially contradictory, sensory requirements. Such leaves would sooner or later depart from the satisfactory compromise condition, either in the direction of too little nutrient (during maturation), or in the direction of too little flavour (during senescence). The shifting pattern of aphid distribution among leaves and plants would then depend on the shifting distribution of leaves where the satisfactory compromise conditions obtained.

Host alternation

In the phenomenon of host alternation we have a particular case of a shifting pattern of aphid distribution: a regular seasonal movement to and from between different kinds of whole plants. The accompanying seasonal changes in the developmental physiology of those plants provide a basis for considering host alternation in the light of the dual discrimination theory. But an additional element has to be reckoned with here: the seasonal changes in the form and probably the physiology of the aphids. The summer forms of *Aphis fabae* are found very largely on its summer hosts and rarely on its winter host. Evidence concerning superficially analogous situations among other insects (e.g. Thorpe & Caudle, 1938) might suggest that these particular aphid forms were equipped with discriminatory reactions against the spindle tree as such, and in favour of summer hosts like beans and sugar beets: that is, against the former's and in favour of the latter's flavour.

The work of Davidson, Mordwilko (1936) and de Fluiter (1949), the observations quoted in Kennedy *et al.* (1950) and the present experiments, have shown that this is not so. Contrary to the view of Hille Ris Lambers (1945: see p. 26), the alienicolae alatae of *A. fabae* will settle and reproduce readily on the winter host, spindle, when its leaves are in a suitable condition. Indeed, other things being equal, it does so more readily on spindle than on a favourite summer host, sugar beet. The fact that even the alienicolous form of the aphid prefers spindle to beet provides some experimental support for the ecologist's and taxonomist's designation of the woody winter host as primary and the summer hosts as secondary, for a given aphid species. On the dual discrimination theory, the flavour of its primary host, spindle, should be preferred by *A. fabae* to that of any other plant; but experiments have yet to be done on the principal secondary host, the broad bean, *Vicia faba* L.

The failure of summer migrants to colonize the spindle tree points, therefore, to some temporary defect in the condition of that host's leaves in the summer. It has been shown that mature leaves are defective, by comparison with young and senescent ones, from the aphids' point of view. It was found that this kind of preference and the fecundity differences associated with it, were strong enough to determine which

leaves, even among those on two different kinds of plant, would acquire the heaviest aphid infestation. Expressed in terms of the dual discrimination theory, the right flavour alone is not enough to hold the aphids; adequate nutrients must also be present.

A study of the relative growth-senescence cycles in winter and summer hosts is still in progress at the time of writing. But it does appear that the leaves of spindle, a shrub, mostly remain mature in summer, and this helps to prevent *Aphis fabae* from colonizing that host at a time when the summer hosts, being herbs, are actively growing, senescing, or both. The forms of aphid which carry out the host selection in spring and autumn may differ behaviourally and nutritionally from the summer forms used in the experiments. But even if the spring migrants have no stronger a preference for summer hosts than have the summer migrants, their re-colonization of the spindle tree in the early summer will be hindered by the ending of the phase of mass growth among its leaves. Even if the autumn migrants have no stronger a preference for the winter host than have summer migrants, their re-colonization of spindle in the autumn will be aided by the onset of mass senescence among its leaves.

If we were to regard host selection as a matter of reactions solely to token substances having no necessary connexion with nutritional requirements, and if we were to study discriminatory reactions only between *kinds* of leaves, then we should be led, with Dethier (1947), to look for the explanation of host alternation entirely within the aphids, that is in seasonal physiological changes causing changes in their host preferences. Hille Ris Lambers' (1934, 1945, and elsewhere) use of 'migration' and 'migratory species' as synonyms for host alternation (heteroecy) and heteroecious species, leads in the same direction. It implies that migration is always from one kind of plant to another, never to the same kind; that again implies that host alternation is due to changes in the host-preferences of the migrants.

Such conceptions of host alternation take no account of the seasonal physiological changes in the plants, which are considered only as fixed, taxonomic units (see Kennedy *et al.* 1950). The aphids are thus assumed to be equipped with discriminatory reactions serving their host-specificity requirements, but with none serving their nutritional requirements, despite the certainly varying food-value of the same host species. And the fact that the primary and secondary hosts of heteroecious aphids are always unrelated botanically, then appears 'rather baffling', as Hille Ris Lambers (1950, p. 142) has said.

The dual discrimination theory escapes these difficulties; and it does not exclude, as an additional possibility, the occurrence of some seasonal changes in the aphids' preferences. On this theory, it is the very fact that the primary and secondary hosts are botanically remote from one another, which facilitates their being united within the host range of one aphid. The botanical difference is connected with a difference in developmental pattern, whereby the one kind of plant offers growing and senescing organs at just those times when the other kind of plant does not; that may have been what made it worth the aphid's while, so to speak, to utilize both kinds of plant during its life cycle.

We are most grateful to Mr A. Ibbotson for his part in the experimental work, to Mr F. J. Anscombe of the Statistical Laboratory, Cambridge, for statistical help, and to Mr A. Judenko and Dr C. G. Johnson of Rothamsted Experimental Station for a translation of Mordwilko's (1936) paper.

REFERENCES

CHIN, C. T. (1950). *Studies on the physiological relations between the larvae of* Leptinotarsa decemlineata Say. *and some solanaceous plants.* Thesis presented to the University of Amsterdam. Wageningen.

DAVIDSON, J. (1921). Biological studies of *Aphis rumicis* Linn. *Bull. ent. Res.* **12**, 81–9.

DETHIER, V. G. (1947). *Chemical insect attractants and repellents.* London.

FIFE, J. M. & FRAMPTON, V. L. (1936). The pH gradient extending from the phloem into the parenchyma of sugar beet and its relation to the feeding behaviour of *Eutettix tenellus*. *J. agric. Res.* **53**, 581–93.

FISHER, R. A. & YATES, F. (1943). *Statistical tables for biological, agricultural and medical research*, 2nd ed. London and Edinburgh.

DE FLUITER, H. J. (1949). On the foodplants of the black bean aphid, *Aphis* (*Doralis*) *fabae* Scop. *Tidschr. PlZ.* **55**, 69–87.

HILLE RIS LAMBERS, D. (1934). Notes on Theobald's 'The Plantlice or Aphididae of Great Britain', II and III. *Stylops*, **3**, 25–33.

HILLE RIS LAMBERS, D. (1945). De Bloedvlekkenluis van appel, *Sappaphis devecta* (Walker). *Tidschr. PlZ.* **51**, 57–72.

HILLE RIS LAMBERS, D. (1950). Hostplants and aphid classification. *Int. Cong. Ent. Stockholm* (1948), **8**, 141–4.

IBBOTSON, A. & KENNEDY, J. S. (1950). The distribution of aphid infestation in relation to leaf age. II. The progress of *Aphis fabae* infestations on pot sugar beets. *Ann. appl. Biol.* **37**, 680–96.

JACOB, F. H. (1945). Notes on the classification of the British species of 'Black Aphides' (Hemiptera, Aphididae). *Proc. R. ent. Soc. Lond.* B, **14**, 102–10.

JACOB, F. H. (1949). A study of *Aphis sambuci* L. (Hemiptera, *Aphididae*) and a discussion of its bearing upon the study of the 'Black Aphids'—Part II. *Proc. R. ent. Soc. Lond.* A, **24**, 99–110.

JONES, M. G. (1947). The bean and beet aphis, *Aphis* (*Doralis*) *fabae* Scop. *The School Science Review*, pp. 357–64.

KENNEDY, J. S. & BOOTH, C. O. (1950). Methods for mass rearing and investigating the host relations of *Aphis fabae* Scopoli. *Ann. appl. Biol.* **37**, 451–70.

KENNEDY, J. S. IBBOTSON, A. & BOOTH, C. O. (1950). The distribution of aphid infestation in relation to leaf age. I. *Myzus persicae* (Sulz.) and *Aphis fabae* Scop. on sugar beet and spindle. *Ann. appl. Biol.* **37**, 651–79.

LAWRENCE, W. J. C. & NEWALL, T. (1948). *Seed and potting composts*, 3rd ed. London.

MORDWILKO, A. K. (1907). Beiträge zur Biologie der Pflanzenläuse, *Aphididae* Pass. *Biol. Zbl.* **27**, 747–67 & 769–816.

MORDWILKO, A. K. (1936). Black bean aphis or beet aphis, *Aphis fabae* Scopoli. [In Russian.] *Plant Prot., Leningrad*, **10**, 69–72.

SALT, G. (1938). Experimental studies in insect parasitism. VI. Host suitability. *Bull. ent. Res.* **29**, 223–46.

STRASBURGER, E. (1930). *Text-book of botany*, 6th English ed. (ed. by W. H. Lang). London.

THORPE, W. H. & CAUDLE, H. B. (1938). A study of the olfactory responses of insect parasites to the food plant of their host. *Parasitology*, **30**, 523–8.

THORPE, W. H., CROMBIE, A. C., HILL, R. & DARRAH, J. (1947). The behaviour of wireworms in response to chemical stimulation. *J. exp. Biol.* **23**, 234–66.

(*Received* 10 *February* 1950)

AGGREGATION IN *APHIS FABAE* SCOP.

I. AGGREGATION ON PLANTS

By ALAN IBBOTSON* and J. S. KENNEDY

Agricultural Research Council Unit of Insect Physiology, Zoology Department, Cambridge

(With 3 Text-figures)

A study of free infestations of *Aphis fabae* Scop. on the leaves of its host plants, spindle and sugar beet, showed that the clustered distribution of the aphids within the boundaries of each leaf was not a matter of chance but involved active aggregation. Experiments in small leaf cages showed that aggregation could take place independently of any differences between portions of a leaf, being brought about by truly gregarious reactions between the aphids. Experiments in which walking aphids were made to encounter other live or dummy aphids fixed to leaves, showed that the gregarious reactions were of at least two kinds. The aphids both probed more often, and remained inserted for longer, near other aphids than apart from them.

It appears that the unequal distribution of aphids among the leaves of a plant is due primarily to intrinsic differences between leaves, but is aided by gregariousness, while the aggregations observed on leaves are due primarily to gregariousness, and only secondarily to differences between portions of a leaf. It is suggested that the primary function of gregariousness is to concentrate the aphids on to the best feeding sites, both among the parts of a plant, and among whole plants; that is, it acts as an aid in host selection, even with aphids which do not aggregate so compactly as *A. fabae*. The functions of compact aggregation and other specialized forms of gregarious behaviour remain to be discovered.

Introduction

Aphids are seldom found distributed uniformly over their host plants. They are usually concentrated to a greater or lesser extent on certain parts of the plants. Two degrees of such concentration may be distinguished. First, the aphids may be denser on certain whole parts of the plant, such as whole leaves, than on other whole parts. In this case, the condition of the favoured parts is often visibly different from that of the other parts, and the concentration may be attributed mainly to the aphids' preferences for feeding, and high fecundity, on plant organs which are in particular stages of growth and senescence (Kennedy, Ibbotson & Booth, 1950; Ibbotson & Kennedy, 1950; Kennedy & Booth, 1951).

Secondly, the aphids may be concentrated into compact groups, separated by bare areas, on leaf or stem surfaces which appear to the eye completely uniform. The term aggregation is here applied only to this second, further degree of concentration, which is clearly exemplified by *Aphis fabae* Scop. (Ibbotson & Kennedy, 1950). Whether such aggregations are due to invisible differences between different

* Now at N.A.A.S. South-West Provincial Centre, Crete Hill, Westbury-on-Trym, Bristol.

parts of the plant surface, or to truly gregarious behaviour on the part of the insects, or to both, can be decided only by experiment. Thus Hille Ris Lambers (1947) showed, by repeatedly dispersing clusters of nymphs on leaves and watching them re-form in new positions, that the aestivating nymphs of *Periphyllis aceris aceris* L. are strongly gregarious. This causes them to aggregate on leaves in a conspicuous and characteristic way.

Similarly, Broadbent (1949) found, in experiments off the plant, that the alate gynoparae of *Myzus persicae* (Sulz.) were truly, if weakly, gregarious. At the same time he counted the free gynoparae on an almond-peach tree, and found that there were more leaves with no aphids at all than would have been expected if the aphids had settled at random. He described this as 'aggregation' and equated it with the phenomenon mentioned by Profft (1939) and others that 'alate *M. persicae* aggregated in groups on peach leaves'. But, in fact, Broadbent's counts, as presented, provided evidence only of concentration of the first degree, on to certain whole leaves, and not of grouping *on* those leaves, or aggregation proper. Among the causes of such concentration on to certain leaves rather than parts of leaves, it seems likely that gregariousness would be overshadowed by the unequal suitability of different leaves, as indeed Broadbent recognized. Gregariousness might be expected to play a larger part in aggregation proper.

The present work deals therefore with the distribution of aphids on single leaves, rather than between leaves. It was designed to demonstrate first that the uneven distribution of *Aphis fabae* on leaves was not a matter of chance but due to active aggregation, and second that there was a truly gregarious component in this aggregation.

Aggregation on spindle leaves

The most striking example of aggregation observed among *Aphis fabae* adults was by alate gynoparous females on some spindle trees in Cambridge in September 1948. It is noteworthy that it was also among gynoparae that Broadbent recorded aggregation in *Myzus persicae*. The *Aphis fabae* gynoparae and their young occurred entirely in dense aggregates at the time of these counts, without a single individual settled by itself. Out of a collected sample of twenty-three of the adult aphids, one was a male, and the rest were gynoparae which, on the criteria used by Kennedy *et al.* (1950), included no *A. euonymi* (Börner & Janisch); nor were there any *A. euonymi* among fifty oviparae collected from the same tree in October.

Fifty leaves and the aggregates they carried were measured on a random sample of shoots, as follows:

Leaves. Length from the junction of lamina and petiole to the tip of the mid-rib; breadth at the widest part of the leaf.

Aphids. Maximum diameter of aggregates; total number of gynoparae in each aggregate.

The leaves were lanceolate in shape and their approximate area was taken as length $\times$ breadth $\times \frac{1}{2}$. Aggregates varied in shape from extended oval to round but

the area which the constituent aphids occupied was always taken as d^2, where d is the maximum diameter of the aggregate. This ensured that any errors would be in the direction of *over*estimating the areas covered by the aggregates.

TABLE 1. *Areas occupied by aggregates of* Aphis fabae *gynoparae and their young in relation to the available area of the spindle leaves colonized*

Aphid aggregates			Leaves			Gynoparae			
						No. of aggregates containing			
Diameter classes (cm.)	No.	Total area (cm.²)	No.	Total area (cm.²)	Total agg. area as % of total leaf area	0	1	>1	Average no. per aggregate
0·0–0·4	40	10·0	30	723·0	1·4	3	30	7	1·4
0·5–0·9	11	11·0	11	297·4	3·9	0	0	11	3·6
1·0+	8	17·0	8	184·8	9·4	0	0	8	4·8
Totals	59	38·0	49	1205·2	—	3	30	26	—

The results are summarized in Table 1, where aggregates are divided into three classes according to their diameters. Each aggregate was on a leaf by itself, except for aggregates of the smallest class, two of which were sometimes found on one leaf. In each class, the total area of leaf covered by the aggregates is given as a percentage of the total area of the leaves on which they were situated, and the number of aggregates of each class which contained 0, 1 and more than 1 gynoparae are also given in the table.

It will be seen that on leaves which carried aggregates even of the largest class, not more than 10 % of the total leaf area was covered by the juxtaposed aphids. The other classes of aggregate covered an even smaller proportion of their total leaf area. Although aphids commonly settle preferentially along the main veins of a leaf, this alone was not what kept them in close mutual proximity on these leaves, since the aggregates were not strung out along the veins, but circular or oval in shape, straddling or even entirely off the main veins.

Had each aggregate consisted of a single gynopara with a number of young, the aggregation might have arisen simply because the young, progeny of the one parent, had not dispersed from where they were born. The table shows, however, that twenty-six of the fifty-nine aggregates of all sizes, and all the aggregates of diameter 0·5 cm. or more, contained more than one gynopara. As no gynoparae were found apart from the aggregates, they must have been much nearer to each other than would have been expected had they been distributed at random on each leaf. Thus the gynoparae themselves were aggregated, and must have been attracted either by each other, or by the young, or by small areas of leaf indistinguishable from others to the human eye, but particularly conducive to aphid settling.

AGGREGATION ON SUGAR BEET LEAVES

Distribution counts showing aggregation among virginoparous populations on the leaves of pot sugar beets have been given elsewhere (Ibbotson & Kennedy, 1950). More critical data were obtained from the following inoculation experiment.

This experiment and the others described subsequently were done in a greenhouse where the temperature varied between 60° and 75° F. (15° and 25° C.), and the humidity was not controlled. The ten plants of var. Bush 'E' used were about 8 in. high and each had about fifteen leaves. The pots were put together in diamond formation on the bench so that their rims were touching in as many places as possible. They were inoculated at the same time, each with fifty adult apterae virginoparae from the mass culture of *Aphis fabae* described by Kennedy & Booth (1950), which were dropped from a specimen tube into the crown of the plant. Measurements were made the next day of the area of each leaf and the distribution of the aphids on it.

This analysis of the results refers only to those leaves on which not less than two, and not more than five aphids had settled, in order to avoid the extremes of aphid density due to differences in suitability of the leaves. The approximate areas of these leaves, calculated as before, and the distances between every aphid and its nearest neighbour on the same leaf, are classified in Table 2.

TABLE 2. *Frequency distribution of distances between apterous adult aphids, and the areas of sugar beet leaf on which they were settled*

Estimated leaf area ($cm.^2$) ...	5–9	10–14	15–19	20–24	25–29	30–34	35+	Total
No. of leaves ...	7	8	6	9	3	2	8	43
Distance between aphids (cm.)								
<0·2	20	19	16	29	14	7	17	122
0·2–0·4	1	—	—	2	—	—	—	3
0·5–0·9	1	—	—	1	—	—	3	5
1·0–1·4	2	—	3	—	—	—	2	7
1·5–1·9	—	2	—	1	—	—	—	3
2·0–2·4	—	2	2	—	—	—	—	4
Total	24	23	21	33	14	7	22	144

It will be seen that, out of the total of 144 aphids which were settled at a density of between two and five per leaf, 122 were within 0·2 cm. of another aphid. They were thus much nearer together than would have been expected if their distribution on the leaves had been random. As before, it follows that the aphids had reacted either gregariously to each other, or independently to the same especially favourable small patches of leaf, although the favoured patches were not visibly distinguishable from the rest.

Aggregation through gregariousness alone

These experiments were designed to show whether aggregation could occur on leaves independently of any preferences the aphids might have for particular parts of a leaf.

The plants used were *Vicia faba* L. grown in 5-in. pots. *V. faba* was chosen because its leaves do not have prominent veins. Each pot contained about twelve plants, and they were used when 8-16 in. tall. The aphids used were wandering adult apterae and alatae from the mass culture cages, which were transferred to experimental leaf-cages. The cage used was a modification of a provisional form of single-leaf cage developed for other work on these insects (Kennedy & Booth, 1950), which enclosed a circular area of leaf under-surface 2 cm. in diam. When in position on a leaf, the cage was completely darkened by two baize-lined metal cover-plates clipped over its top and bottom. Unfurled terminal leaves, of which the venation on one side of the midrib was a mirror image of that on the other, were selected for use. Several layers of paraffin wax were painted along the midrib on the underside of the leaf, thereby preventing the aphids from settling to feed there. The cage was clipped on to the leaf in such a way that equal areas of lamina were exposed on either side of the waxed midrib.

The aphids were caged by dropping them from a specimen tube into the container-lid, which was then quickly re-attached to the main body of the cage already fixed on the leaf. Five cages were used simultaneously for each experiment. Ten aphids were put into each cage. In each of the first three experiments the five cages were filled with fresh aphids fifteen times each, and opened 3 hr. after each filling when the number of aphids settled on each side of the midrib was recorded. Apterous adults were used in two experiments and alate adults in the third.

Table 3. *Successive distributions of settled aphids between two equal areas of a caged leaf when the aphids were introduced on one side of the cage, indicated by the italicized figures*

No. of cage ...	1		2		3		4		5	
Side of cage ...	L.	R.	L.	R.	L.	R.	L.	R.	L.	R.
Distributions	5	*1*	*1*	8	3	7	6	2	6	*3*
	8	2	8	*2*	*5*	2	2	*8*	*3*	7
	3	7	*3*	7	2	*8*	7	3	8	*2*
	2	6	2	*8*	*0*	9	8	*1*	*8*	2
	5	*4*	6	2	3	*4*	*2*	6	1	7
	7	1	6	*4*	*3*	6	5	*3*	6	3
	7	*3*	7	2	3	7	7	3	4	6
	6	4	2	*8*	*3*	7	1	*8*	*4*	6
	3	7	6	4	2	6	*4*	6	7	*2*
	2	6	1	9	7	2	7	*2*	*8*	2
Total	48	41	42	54	31	58	49	42	55	40

When dropped into the cages, the aphids often fell asymmetrically on the floor of the cage lid rather than as intended, in its centre. If they had all climbed the nearest point of the cage wall and inserted their mouth-parts into the nearest portion of leaf, then, on the average, a greater proportion of them would have settled on one side of the midrib than if they had been distributed uniformly over the lid floor initially. This would have produced a false impression of aggregation since, over a number of tests, the variance of the distribution of the aphids between the two sides would have been greater than expected with random settling. In two further experiments therefore the initial distribution of the aphids was carefully controlled. Adult apterae were introduced into five cages ten times, and the cages were opened for counting 30 min. later. In the first experiment the ten aphids were deliberately dropped together in a ball into the cage lid in such a way that, when the lid was attached to the cage, the aphids were all under one side of the midrib, the sides being alternated in consecutive tests. In the other experiment the aphids were dropped into the lid simultaneously from two specimen tubes so that when the lid was attached to the cage there was a ball of five aphids beneath each side of the midrib. The results are given in Tables 3 and 4.

TABLE 4. *Successive distributions of settled aphids between two equal areas of a caged leaf when the aphids were introduced symmetrically into the two sides of the cage*

No. of cage ...	1		2		3		4		5	
Side of cage ...	L.	R.	L.	R.	L.	R.	L.	R.	L.	R.
Distributions	4	6	7	1	1	8	2	8	7	3
	5	5	6	4	8	2	5	5	8	2
	9	0	4	6	2	7	3	7	2	7
	1	7	4	4	3	6	5	5	3	6
	4	6	3	7	8	2	6	3	2	6
	7	3	6	2	6	4	6	1	6	3
	2	7	7	3	3	7	10	0	1	5
	7	3	8	2	7	2	2	6	6	3
	6	1	1	6	6	3	7	1	2	6
	1	6	3	4	2	7	1	6	3	6
Total	46	44	49	39	46	48	47	42	40	47

If the aphids settle independently of one another, then the expected variance e of the proportion of aphids found settled on one of the two sides of any one caged leaf, in a series of tests, will be given by

$$e = \bar{x}(1-\bar{x})/n,$$

where $\bar{x}$ = the mean proportion settled on one side, and n = the mean total number settled at each reading, which is obtained from:

$$\frac{1}{n} = \frac{1}{n_1} + \frac{1}{n_2} + \frac{1}{n_3}, \ldots, + \frac{1}{n_N} \Big/ N,$$

where N = the number of readings.

The experimentally observed variance o is given by:

$$o = \Sigma(x - \bar{x})/N - 1,$$

where x = the proportion settled on one side at each reading,

$\bar{x}$ = the mean proportion settled on one side,

and N = the number of readings.

The ratio between the two variances, o to e, indicates the degree to which the aphids were observed to gather together on one side of the midrib, by comparison with what we should expect if they had no gregarious tendency. The values of o/e, derived both from the uncontrolled- and from the controlled-introduction experiments, are shown in Table 5, together with their levels of significance (Fisher & Yates, 1948). Table 5 shows that over a succession of readings, on twenty-one out of twenty-five different caged leaves, the observed variance of the distribution of aphids between the two equal areas of leaf was significantly greater than would be expected if settling were random. The observed variance was somewhat greater than the expected variance in all twenty-five cages. Thus the distribution of the apterous and alate adults between the two sides of the cages must have been influenced by gregarious reactions among the aphids. The results from the controlled-introduction experiments, given in the last two columns of Table 5, show that the method of introducing the aphids had little effect on the degree of their aggregation. Evidently the aphids preferred to settle near other aphids rather than by themselves.

TABLE 5. *Ratios of observed to expected variances of the distribution of apterous and alate adults between two equal areas of a leaf*

Cage no.	Uncontrolled introductions: ratios based on fifteen readings at 3 hr. intervals			Controlled introductions: ratios based on ten readings at ½ hr. intervals (apterae)	
	Apterae		Alatae	Alternate one-sided introductions	Equal, two-sided introductions
1	2·25*	1·80†	2·42*	2·25†	3·23†
2	2·32*	1·70†	2·91*	3·23*	1·96†
3	1·55	1·50	2·39*	2·04*	2·78*
4	1·61	2·10*	2·81*	2·76*	3·33*
5	2·60*	1·53	2·30*	2·36†	1·99†

* Significant at 1 % (> 2·07 for 3 hr., > 2·41 for ½ hr. readings).
† Significant at 5 % (> 1·69 for 3 hr., > 1·88 for ½ hr. readings).

The degree of aggregation in these cages was much less than that observed among free aphids on the plants. That does not mean that gregarious reactions play only a small part in bringing about the aggregation of free aphids. One reason why natural aggregations are more striking than those in the leaf cages, is that the former include large numbers of nymphs. Since nymphs are much less inclined to wander than adults, and tend to remain where they have been born (Kennedy *et al.* 1950; Ibbotson & Kennedy, 1950), the degree of aggregation of the whole population of

aphids, although mainly dependent on the gregarious reactions of the wandering adults, becomes greatly magnified on the free plant. Again, the nearly simultaneous arrival on a given area of leaf of ten aphids coming from all points round its periphery, which must have occurred in the leaf cages, would easily lead to two or more separate aggregation nuclei coming into being at the same time. On the other hand, aphids arriving on a free leaf one after the other and along one route, the petiole, would be more likely to build up an aggregate cumulatively on one original nucleus. There were also other conditions of the leaf-cage experiments militating against the maximum display of gregariousness, such as the darkness which precluded mutual visual reactions (see Ibbotson, in preparation).

GREGARIOUS REACTIONS

These experiments were designed to confirm the existence of truly gregarious behaviour among these aphids, by arranging meetings between individuals on a leaf surface, and recording their reactions.

Method

Wandering apterous adults were taken from the mass culture, and induced to walk up the midribs of pot sugar beet leaves. These aphids have a noticeable tendency to walk along prominent veins rather than across the smooth surface of a leaf so that once set on the midrib they usually kept on it as long as they continued walking. This tendency was made use of also to bring them on to the midrib in a repeatable and reasonably unexcited state. The aphids will walk steadily along any ridge the width of which fits the normal span of their legs so that the tarsi comfortably grasp its edges. A 'cat-walk' 2 mm. wide, joined to a platform 1·5 cm. square, was therefore cut from thin card. Fig. 1 shows how the platform was impaled on a wire in

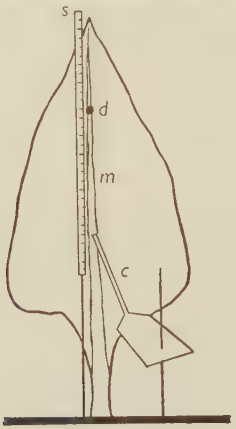


Fig. 1. Sketch of arrangement for testing the responses of walking aphids to a fixed decoy. *c*, 'cat-walk' along which the aphids reach the leaf midrib *m*, and pass on up it to the decoy at *d*, the positions of their probes being recorded on the scale *s*.

front of the underside of a leaf held upright on the plant by a clip at its apex. The platform was arranged so that the cat-walk led upwards at an angle of about 60° with its tip touching the leaf's midrib. A scale drawn in 0·25 cm. divisions was supported parallel to the midrib and about 0·5 cm. from it. Aphids were put on the platform one by one and guided to the foot of the cat-walk with a fine camel-hair brush.

Records were kept of the positions along the midrib at which the aphids probed as they moved from the end of the cat-walk up to the apex of the leaf. The leaves used had all carried a small aggregate of one or two apterous adult *Aphis fabae* and their young settled across the mid-rib near the tip of the leaf. These aphids were all removed immediately before the tests began. Two series of tests were done, in which the aphids were presented with four different situations in succession:

Series A

1. Leaf bare.
2. One live apterous adult fixed by a spot of spirit varnish in the position of the original aggregate, as a decoy.
3. Cat-walk raised so that its tip came higher on the midrib than for 2, with the decoy aphid still in position.
4. Decoy removed leaving the leaf bare again, and the cat-walk lowered to its first level.

Series B

1. Leaf bare.
2. Small black stone of the same size and shape as the body of an apterous adult, fixed in the position of the original aggregate, as a decoy.
3. Stone removed and replaced by live aphid decoy.
4. Decoy removed leaving the leaf bare again.

In both series, all the aphids of a batch were first presented with test-situation 1, then the test-situation was changed to 2 and all the aphids presented again, and so on. Two batches each of fifteen aphids were subjected to each series of tests. In series B, not only the position but also the duration of each probe was recorded, in one of three classes: less than 1 min.; 1–2 min.; and more than 2 min.

Results

Aphids probed into the leaf in a very characteristic way. Each checked its forward movement, erected its proboscis and with the antennae pointing forwards and still vibrating, made one or two jabs at the leaf surface, finally bringing the proboscis to rest on it and inserting the stylets. The antennae continued vibrating forwards for a few seconds after that, but were then laid back over the body and became quite motionless. When the aphid was about to move on again the antennae were swept forwards and began vibrating before the proboscis was lifted from the leaf. The proboscis was laid back between the coxae as the aphid moved off.

The term *probe* is used here for the completed action of inserting the mouth-parts and bringing the laidback antennae to rest. Tests showed that when the proboscis

was held erect against the leaf surface for some seconds, it could be assumed that the stylets were inserted. When such completed probes occurred upon meeting a decoy object, the first stage, arrestation, often appeared without actual contact with the object, and was followed by a variable amount of fidgeting about close to it, often including semi-crab-wise movement down the side of the midrib. The probe

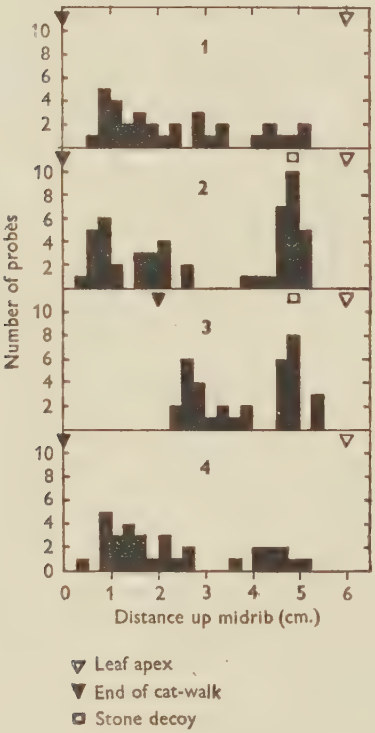


Fig. 2. Distribution of probes made by aphids walking up the midrib of a sugar-beet leaf to which a decoy aphid was attached.

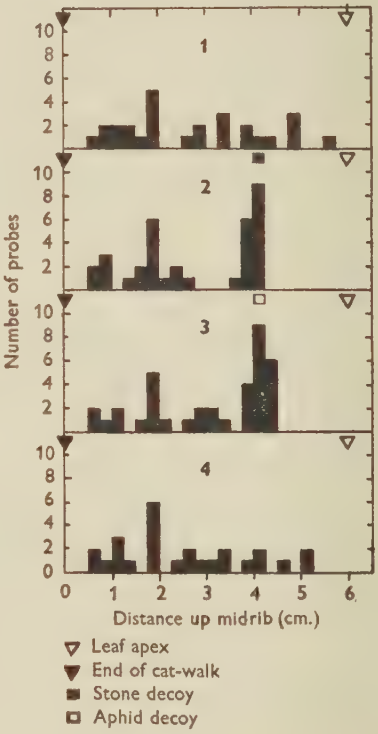


Fig. 3. Distribution of probes made by aphids walking up the midrib of a sugar-beet leaf to which stone and aphid decoys were attached in turn.

itself occurred sometimes before and sometimes after the probing aphid had made contact with the object.

The distribution of probes along the midrib is shown for test-series A and B in Figs. 2 and 3 respectively. The total numbers of probes made within 0·5 cm. of the decoy aphid or stone, and the total number made elsewhere along the midrib, are summed in Table 6, while Table 7 shows the durations of the probes recorded in test-series B. The aphids probed more often in the vicinity of a live aphid or stone dummy, than at any other point along the midrib. This was not because they had

a tendency to probe after walking a particular *distance*, for they did not concentrate their probes at the same distance from their starting-point in the absence of a decoy, and when the cat-walk was brought nearer to the decoy they still concentrated their probes at the decoy (Table 6, A 3). And, since the decoy position had been occupied by a group of aphids before the tests began, but did not attract probes when there was no decoy there, it is evident that the group had not left external traces on the leaf sufficient to induce other aphids to probe preferentially in that place (cp. Broadbent, 1949).

TABLE 6. *Number of probes by aphids when walking up the midrib of a beet leaf with and without attached decoy objects*

Test series	Decoy situation	No. of probes: Within 0·5 cm. of decoy position	Elsewhere	Total
A	1. None	6	27	33
	2. Aphid at 5 cm.	23	28	51
	3. Aphid at 2 cm.	17	18	35
	4. None	6	26	32
B	1. None	4	23	27
	2. Stone at 4 cm.	16	18	34
	3. Aphid at 4 cm.	19	17	36
	4. None	4	23	26
Totals	None	20	98	118
	Aphids and stones at various distances	75	81	156

TABLE 7. *Durations of probes in test series B (Table 6)*

Decoy situation	No. of probes: Within 0·5 cm. of decoy position and lasting (min.) <1	1–2	>2	Total	Elsewhere and lasting (min.) <1	1–2	>2	Total
1. None	3	1	0	4	15	5	3	23
2. Stone at 4 cm.	11	3	2	16	12	4	2	18
3. Aphid at 4 cm.	3	4	12	19	15	2	0	17
4. None	1	3	0	4	16	6	0	22
Totals, none	4	4	0	8	31	11	3	45
Totals, aphids and stones at 4 cm.	14	7	14	35	27	6	2	35

It is not known whether probes made elsewhere than at the decoy were usually more or less spontaneous, or were evoked in much the same way as those at the decoy, by some immediate environmental stimulus. In test-series B (Fig. 3) there was a concentration of probes at a point 1·75 cm. up the midrib where two large veins branched off, as it happened, on both the leaves used. The aphids grasped the sides

of the midrib as they moved up it, and the large diverging veins guided their feet further and further apart, until they had to break step and pause while regaining their alinement on the central midrib. Strictly speaking, neither these probes nor those at the decoys could be described as evoked directly by their external cause. The common effect of the different external stimuli was first to arrest the aphid, and that seemed to be the immediate cause of probing, which was thus released rather than evoked by the external stimulus.

The totals in Table 6 show that the increase in the number of probes at a given point on the midrib, brought about by placing a decoy there, was both absolute and relative. It was partly due to the interpolation of extra probes at that point, and partly to a reduction in the number of probes made along the stretch of midrib before the decoy site, suggesting that the aphids were responding to the decoy well before they reached it. Table 7 shows that, having probed, aphids remained inserted much longer, on the average, near another aphid than they did near a stone decoy. Although they probed by the black stone as often as they probed by another aphid, the aphids probing by stones did not stay so long. They withdrew their stylets and moved on again as soon as they did after probing elsewhere along the midrib where there was no decoy.

DISCUSSION

The results show that *Aphis fabae* aggregates in the free state, and that this is in part a truly gregarious phenomenon. We are now in a position to assess, in a preliminary way, how far gregariousness on the one hand, and the unequal susceptibilities of different parts of the plant on the other, contribute to the uneven distribution of *A. fabae* on its host plants. There is considerable evidence that the primary cause of concentration on to certain whole leaves is the fact that the aphids remain feeding for longest, and multiply most rapidly, on leaves which are in certain stages of growth and senescence (Kennedy *et al.* 1950; Ibbotson & Kennedy, 1950; Kennedy & Booth, 1951). But it follows from the present work that the longer an aphid remains feeding in one place, the more likely it is that another aphid will probe in the same place. Thus the number of probes made into suitable places on the plant will be increased, and the aphids will be more likely to settle in places which are more suitable for them. In this way gregariousness is probably an adjuvant cause of concentration, the primary cause being local differences presented by the plant and the aphids' consequent preferences for certain parts, such as leaves, of a particular physiological age.

Within the confines of single leaves, local differences presented by the plant can still contribute to the uneven distribution of the aphids. One obvious kind of local differentiation within the confines of a leaf is the system of veins. Aphids are often found concentrated along the main veins, where they feed for preference in the phloem (Davidson, 1923; Tate, 1937; Roberts, 1940). Also, walking aphids move preferentially along prominent veins (p. 72) and in consequence encounter one another, with the opportunity of reacting gregariously, more often than they would

if the leaf's surface were smooth. Nevertheless, the aphids occur in compact, roughly circular aggregates lying across or out of contact with the main veins; the aphids are not alined along the veins (p. 67). They become so alined at times, but only when the compact aggregates break up and the aphids are leaving a leaf. Thus venation seems to contribute indirectly to the unequal distribution of *A. fabae* within the boundaries of single leaves, but gregariousness is the primary cause of the clustered arrangement of aphids there.

The formation of compact aggregates is so striking a result of gregariousness in *A. fabae*, that its role in the life of the species merits further study. *A. fabae* individuals not only cluster together, but also jerk their bodies in concert, as do various other aphids: a convulsive upwards and sideways 'kick' of body and legs together, made while pivoting on the firmly embedded mouth-parts. Our impression is that amongst other things such kicking is sometimes, although by no means always, attributable to disturbance by parasites and predators. Thus an adult coccinellid—but not an ant—running over a colony of *A. fabae* will start them kicking in this peculiar manner. Although cursory inspection suggested that most of the aphids' enemies were not deterred by the kicking, it did appear to hinder oviposition by some *Aphelinus*-like parasites, which terminated their pre-ovipository manœuvres toward and moved away from an aphid that moved. Some form of gregarious interaction is indicated by the way the proportion of aphids in a colony, which are taking part in an outburst of kicking, builds up to a peak and dies away again. Their jerks often show a remarkable degree of synchronization without any obvious external changes to provide a common stimulus (cp. Taylor, 1950).

Whatever may be the significance of forming *compact* aggregates, however, it cannot be assumed that this is the most important effect of gregariousness, even for *Aphis fabae*. Mutual *visual* stimulation plays an important part in the gregarious behaviour of *A. fabae* (Ibbotson, in preparation), and its black colour may make it more efficient in this respect than *Myzus persicae*. But *M. persicae* is also gregarious (Broadbent, 1949) and the fact that it does not form dense clusters like *Aphis fabae* is not satisfactory evidence that its gregarious responses are, in themselves, any weaker. In unpublished experiments, walking *Myzus persicae* individuals responded, by probing, when they encountered a stationary individual, much as *Aphis fabae* does. Lack of compact aggregation by *Myzus persicae* may be due mainly to the greater restlessness of this species. This might prevent a walking individual from coming to rest at once, immediately against an already settled fellow which it met and responded to gregariously, without preventing the responder from settling down somewhere near its fellow, say on the same leaf. *Aphis fabae* also failed to aggregate compactly when the individuals became restless (above). Thus failure to aggregate compactly need not prevent gregarious responses from helping to concentrate the aphids on to the most suitable parts of the plant. This may be the primary effect or function of gregariousness, for as far as that sort of concentration is concerned, *Myzus persicae* is not noticeably less efficient than *Aphis fabae* (Kennedy *et al.* 1950).

Gregariousness could equally assist in retaining, and so concentrating migrant aphids on suitable whole plants, that is in specific *host* selection. The importance of this function of gregariousness will be the greater, if there is no distant attraction of aphids to their specific hosts, selection between suitable and unsuitable hosts being exercised after rather than before alightment on the plants (Kennedy, 1950). Perhaps it is because the hosts suitable for autumn migrants are woody plants, often few and far between, that the most striking field examples of aggregation by both *Aphis fabae* and *Myzus persicae*, are among the autumn migrants on their winter hosts, these migrants having, perhaps, particularly well-developed gregarious tendencies.

This work was done while the first author held a scholarship from the Agricultural Research Council, and his thanks are due to Dr V. B. Wigglesworth for granting him facilities with the Unit of Insect Physiology.

REFERENCES

BROADBENT, L. (1949). The grouping and overwintering of *Myzus persicae* Sulz. on *Prunus* species. *Ann. appl. Biol.* **36**, 334–40.

DAVIDSON, J. (1923). Biological studies on *Aphis rumicis* Linn. The penetration of plant tissues and the source of food supply of the aphids. *Ann. appl. Biol.* **10**, 35–54.

FISHER, R. A. & YATES, F. (1948). *Statistical Tables for Biological Agricultural and Medical Research*. 3rd ed. viii + 112 pp. London and Edinburgh.

HILLE RIS LAMBERS, D. (1947). Notes on the genus *Periphyllus* v.d. Hoeven (Hom., Aph.). *Tijdschr. Ent.* **88**, 225–42.

IBBOTSON, A. Aggregation in *Aphis fabae* Scop. II. Behaviour analysis. (In preparation.)

IBBOTSON, A. & KENNEDY, J. S. (1950). The distribution of aphid infestations in relation to leaf age. II. The progress of *Aphis fabae* infestations on pot sugar beets. *Ann. appl. Biol.* **37**, 680–96.

KENNEDY, J. S. (1950). Applied migration and the spread of plant viruses. *Nature, Lond.*, **165**, 1024.

KENNEDY, J. S., IBBOTSON, A. & BOOTH, C. O. (1950). The distribution of aphid infestation in relation to leaf age. I. *Myzus persicae* (Sulz.) and *Aphis fabae* Scop. on sugar beets and spindle trees. *Ann. appl. Biol.* **37**, 659–79.

KENNEDY, J. S. & BOOTH, C. O. (1951). Host alternation in *Aphis fabae* Scopoli. I. Feeding preferences and fecundity in relation to the age and kind of leaves. *Ann. appl. Biol.* **38**, 25–64.

PROFFT, J. (1939). Ueber Fluggewohnheiten der Blattläuse in Zusammenhang mit der Verbreitung von Kartoffelvirosen. *Arb. phys. angew. Ent.* **6**, 119–45.

ROBERTS, F. M. (1940). Studies on the feeding methods and penetration rates of *Myzus persicae* Sulz., *Myzus circumflexus* Buckt. and *Macrosiphum gei* Koch. *Ann. appl. Biol.* **27**, 348–58.

TATE, H. D. (1937). Method of penetration, formation of stylet sheaths, and sources of food supply of aphids. *Iowa State Coll. J. Sci.* **2**, 185–206.

TAYLOR, L. R. (1950). Synchronous movement of *Aphis fabae* Scop. (Hem. Aphididae). *Ent. mon. Mag.* **86**, 52.

(*Received* 22 *June* 1950)

THE POTATO TUBER NEMATODE, *DITYLENCHUS DESTRUCTOR* THORNE, 1945; THE CAUSE OF EELWORM DISEASE IN BULBOUS IRIS

By J. BASIL GOODEY

Nematology Department, Rothamsted Experimental Station, Harpenden

(With Plates 1 and 2)

For about twenty-five years a fairly widespread disease of bulbous iris has been known, the cause of which has always been attributed to a biologic race of the stem eelworm *Ditylenchus dipsaci* (Kühn, 1857), Filipjev, 1936.

Thorne (1945) showed that the nematode causing rot in potato tubers is different from *D. dipsaci* and named it *D. destructor*. This nematode has since been reported from *Mentha arvensis* L. by Hurst (1948) and from *Sonchus arvensis* L. by Goodey & Goodey (1949).

Examination of nematodes from diseased iris bulbs showed them to possess rounded tail tips and six incisures on each lateral field; characters by which *Ditylenchus destructor* is distinguished from *D. dipsaci*.

Cross-inoculation experiments showed that the eelworm causing disease in potato tubers would invade and set up characteristic symptoms in iris bulbs and, in the opposite direction, the eelworm responsible for disease in iris bulbs would give rise to characteristic symptoms in potato tubers. Transfer was also effected from potato and iris to *Mentha arvensis* and from iris to *Sonchus arvensis*.

The history of the disease in bulbous iris is briefly reviewed and the biology of *Ditylenchus destructor* discussed and compared with that of *D. dipsaci*.

The conclusion that *D. destructor* is the nematode causing eelworm disease of bulbous iris has been reported earlier (Goodey, J. B. 1950).

History of the disease in iris

Rot in the bulbs of an unnamed species of iris assumed to be caused by *Ditylenchus dipsaci* was first reported by van Poeteren (1925) in Holland. The Spanish iris, *Iris xiphium* was recorded as a host in Great Britain by T. Goodey (1929) though it was uncertain where the bulbs had been grown. These cases were observed by both van Poeteren and Goodey in 1924. Some years later Tschaen (1932) found the disease in two more species of iris, *I. filifolia* and *I. tingitana* from the French Mediterranean area. Amongst the remedies suggested by him to control the disease was the use of a crop rotation including, especially, the potato. In the same year the disease was first recognized in the United States on *Iris* sp. (Anon. 1932). The next year Steiner & Buhrer (1933*a*) published records of thirty-eight cases of infestations on bulbs intercepted by the Quarantine Division of the United States Department of Agriculture between 1928 and 1932, in which English iris, *I. xiphioides*, was named

for the first time as a host. Although Tschaen remarked, in passing, that both narcissi and hyacinths suffered considerably from attacks by *Ditylenchus dipsaci*, Steiner and Buhrer were the first to give any consideration to the possible source of the disease. They pointed out that though there was some yellowing of the leaves, no worms could be found in them, nor did the leaves bear blister-like swellings, the so-called 'spikkels'. They said that the infestation usually began on the side of the bulb, appearing as a yellowish spot or streak which gradually spread, becoming brown and then grey while the bulb scale became spongy. Later more general rot supervened and the bulbs usually died after five or six months' infestation. They tried to infest narcissus and onions from the iris material but failed to do so. The same authors (1933*b*), and Anon. (1935*a*), reported *D. dipsaci* infestation in *Iris tingitana* var. Wedgewood.

Hodson (1934) gave a detailed description of symptoms both in the dormant bulbs and during growth for English iris vars., Sky Blue, Blue King and Bridesmaid, and for Dutch iris vars. Wedgewood and Imperator. In most cases the bulbs had been imported from Holland. He suggested that the infestation in Imperator might be of narcissus origin as the bulbs were grown on ground which had previously carried narcissus, though he failed to confirm this when he carried out experiments to test the possibility. He pointed out, too (Hodson, 1935), the severity with which English iris was attacked and was of the opinion that the iris race of the parasite was unable to damage hyacinths and narcissi. Another new host, *I. histrioides major*, reputedly of British origin, was reported in 1935 from America (Anon. 1935*b*). The disease also occurred in Canada on *Iris* sp. var. Hart Nibbrig, as reported by Hastings (1935). Hastings claimed to have effected a transfer from narcissus to iris and thence to seedlings of Barks barley in which lesions similar to those produced by attack of the narcissus strain of *Ditylenchus dipsaci* were said to be caused.

Hastings & Bosher (1936), remarking on the uncertainties in diagnosing the disease, said that it was easier if the tunic was stripped from the bulb. Discoloured spots or streaks leading from the bulb base could then be seen which, they said, were specific symptoms. The tunic itself and the lowest inch or so of the flower stalk, enclosed by the tunic, might also be infested; this infestation showed as yellow, black or grey streaks extending into the basal plate and from there initiating infestation in the daughter bulbs. In a further study of the disease, Newton, Hastings & Bosher (1937) said that the normal route of infestation was through the basal plate, that discoloration round the sunken base was more common and characteristic than streak symptoms and that not all the daughter bulbs necessarily became infested. Hastings & Bosher (1938) investigating the populations infesting iris bulbs, showed that Imperator (Dutch) was less susceptible than Supreme (Dutch) and Prince Albert (English); this being correlated with the relative ability of the nematodes to multiply within the tissues.

In the following list emended from T. Goodey (1940), the species of iris with the authority for the first record of attack are given:

Iris filifolia Bois.	Tschaen, 1932.
I. histrioides major Dykes.	Anon. 1935.
I. tingitana Bois. & Reut.	Tschaen, 1932.
I. tingitana var. Wedgewood	Anon. 1935.
I. xiphioides Ehrb.	Steiner & Buhrer, 1933.
I. xiphium L.	Goodey, T., 1929.
Iris sp.	Poeteren, van, 1925.

There are some inconsistencies in the literature with reference to the Dutch race of iris which may be called either *I. tingitana*, or *I. xiphium*, hybrids. The parentage of the Dutch race is apparently very mixed and includes *I. Boissieri* Henriq., as well as the two species already mentioned. It might be better, therefore, to refer to this race merely as the Dutch iris, without any further attempt at scientific precision.

For the sake of completeness it may be mentioned here, that the successful control of the pest by warm-water treatment of the bulbs is possible and is dealt with by Staniland (1935), Staniland & Barber (1937) and Hastings, Bosher & Newton (1939).

Transfer experiments

1. *Preliminary work*

Iris bulbs were obtained from a British firm in December 1947 and an experiment was set up attempting the transfer of *Ditylenchus destructor* from potato to iris (Table 1). Four varieties of iris were used, three of them, Rembrandt, White

Table 1. *Transfer of* D. destructor *from potato to iris*

Iris variety	Bulbs planted	Treatment	Bulbs harvested: Clean	Bulbs harvested: Infested	Comments
Rembrandt (D)	8	Control	10	0	—
Rembrandt (D)	8	Inoculated pot	15	11	—
Rembrandt (D)	8	Flap	2 small	0	Majority of bulbs rotted away
White Excelsior (D)	8	Control	13	15	—
White Excelsior (D)	8	Inoculated pot	6	10	—
White Excelsior (D)	8	Flap	0	0	All rotted away
Wedgewood (D)	8	Control	42	0	—
Wedgewood (D)	8	Inoculated pot	24	0	—
Wedgewood (D)	8	Flap	8 small	0	Mostly rotted away
Mont Blanc (E)	8	Control	46	0	—
Mont Blanc (E)	8	Inoculated pot	0	0	All rotted away
Mont Blanc (E)	8	Flap	0	0	All rotted away

D = Dutch. E = English

Excelsior, and Wedgewood were Dutch and the fourth was an English one, var. Mont Blanc. Eight bulbs of each variety were potted in John Innes compost just below a liberal layer of pieces of eelworm-diseased potato. A second set of bulbs was inoculated by the insertion of pieces of diseased material beneath a flap of tissue made by a cork-borer and then sealed over with adhesive cellulose tape (Baker, 1948). These and a control set of bulbs were also planted in John Innes compost. The second

set of bulbs, almost without exception, rotted away so the flap method of inoculation was discontinued for bulbs. The pots were kept in a cool greenhouse, the plants allowed to flower and an examination of the bulbs finally made at the end of August 1948. Taking the control bulbs first; those of Rembrandt, Wedgewood and Mont Blanc were quite clean, but the controls of White Excelsior showed infestation in fifteen out of twenty-eight bulbs. In the set of inoculated pots, Rembrandt showed infestation in eleven out of twenty-six bulbs and White Excelsior, in ten out of sixteen; Wedgewood showed none, whilst all the bulbs of Mont Blanc had rotted away. It was concluded (1) that the stock White Excelsior had been infested when bought and that the other stocks used might also be suspect; (2) that Wedgewood was not a very susceptible variety; (3) that the infestation had probably been transferred from potato to Rembrandt; and (4) that Mont Blanc was probably a very susceptible variety and under the conditions of the experiment had succumbed to the severity of the attack. The results in terms of varietal susceptibility were in broad agreement with those of previous workers in that the English iris showed more marked symptoms of attack than the Dutch. The conclusions were not clear cut and could only be used as pointers to further investigations.

2. *Iris to potato*

An experiment was set up on 31 August 1948, attempting transfer of the nematodes from iris to potato (Table 2). Using a method devised by Baker (1948), chiropodists'

Table 2. *The transfer of* D. destructor *from iris to potato*

Tuber nos.		Source of inoculum	Origin of nematodes
1	9	White Excelsior Control from Exp. 1	Iris
2	10	White Excelsior Control from Exp. 1	Iris
3	11	White Excelsior previously infested from potato, Exp. 1	Potato and iris?
4	12	White Excelsior previously infested from potato, Exp. 1	Potato and iris?
5	13	White Excelsior previously infested from P. de Ridder	Iris
6	14	Rembrandt previously infested from potato, Exp. 1	Potato
7	15	P. de Ridder	Iris
8	16	P. de Ridder	Iris

Nos. 1–8 untreated tubers. Nos. 9–16 tubers treated with sodium thiocyanate.

corn pads containing portions of diseased iris material resting on small pieces of damp filter-paper were stuck over the eyes of small potatoes, var. Duke of York. The inoculum was from various sources with different histories, some being iris material previously infested from potato. Thus in these cases the eelworm had as hosts, potato, then iris and finally potato again. A similar set of inoculations was made on tubers which had been treated with 3 % sodium thiocyanate solution to break dormancy. Both sets of tubers were placed in screw-top jars in an incubator at 24° C. The tubers treated to break dormancy sprouted first and on 2 October 1948 one had a shoot about $\frac{1}{2}$ in. long. The flesh at the base of this shoot was soft and

slightly cracked. This tuber had been inoculated with material from iris P. de Ridder and numerous specimens of *Ditylenchus destructor* in all stages from egg to adult were present in the soft flesh. By 19 November 1948 another of the tubers showed symptoms of infestation.

There was no doubt that nematodes from diseased bulbous iris had set up infestation in potato tubers with the production of typical symptoms.

The remainder of the tubers were planted in large pots of John Innes compost and allowed to grow on in a warm greenhouse. When examined in the third week of April 1949, a total of thirty-eight tubers had been produced from fifteen planted and of these eighteen were well infested with *D. destructor*.

3. *Iris to mint*

On 20 September 1948 an experiment to set up infestation in *Mentha arvensis* with nematodes from iris was begun (Table 3). Four pieces of mint rhizome were

TABLE 3. *The transfer of* D. destructor *from iris to mint*

Pot nos.		Treatment	Source of inoculum	Result
1	5	Control	Wedgewood iris	Clean
2	6	Pad	Wedgewood iris	All infested
3	7	Pad	Wedgewood iris	
4	8	Inoculated pot	Wedgewood iris	

inoculated with pieces of diseased iris bulb, var. Wedgewood, originating from Guernsey. The diseased material was placed in the cavity of a corn pad on moist filter-paper and the node of a rhizome enclosed between this and another pad adhering face to face. The rhizomes were then potted in John Innes compost. Two more pots contained rhizomes planted below a layer of diseased iris bulb material and two more were set up with clean rhizomes as control. The pots were kept in a warm greenhouse and growth proceeded well. One pot examined about 5 weeks later, 2 October 1948, containing a pad inoculation, showed two small lesions on the rhizome enclosed between the pads though no eelworms were present. They were found, however, in the brownish broken end of the rhizome and had no doubt migrated through the soil and attacked the tissues at this unprotected spot. Most of the other pots were examined on 19 January 1949, infested lesions being present on the rhizomes, but one pot was allowed to remain till 17 August 1949, when it was found that the infestation was still present, numerous larvae being teased out from several lesions. The control rhizomes remained quite clean.

4. *Cross-infestation experiment*

A large experiment using 136 pots was set up on 18, 19 and 20 October 1948 to test as many cross-infestations as possible. The plants used were as follows: seed potatoes from a home-grown 1948 crop of Duke of York; pieces of rhizomes of *Mentha arvensis* and *Sonchus arvensis* dug from local fields; home-grown narcissus bulbs, var. King Alfred; iris bulbs bought from firms in Britain and Holland.

Every specimen used was carefully examined to make sure it was quite free from prior infestation. The potatoes, mint and daffodils were quite clean. The iris bulbs, on the other hand, were a mixed lot. Each bulb had its tunic stripped so that any disease symptoms could be plainly seen. Of the two varieties from the British firm, Wedgewood (Dutch) was clean but Mont Blanc (English) was quite heavily infested. Twelve varieties of iris were obtained from Holland; two Dutch varieties, White Excelsior and Rembrandt and five Spanish varieties, La Nuit, Giant, King of the Blues, L'Innocence and Le Megol, were quite free from infestation, but practically every bulb of five English varieties, Sarah Bernhardt, Mansfield, Czar Peter, Queen of the Violets and Queen of the Blues, was severely infested with eelworm. This diseased material was used in the experiment for inoculation purposes. All specimens used were planted below a layer of diseased material in pots of John Innes compost. The potatoes were grown in $9\frac{1}{2}$ in. and everything else in $4\frac{1}{2}$ in. pots. The whole experiment was kept in a greenhouse heated sufficiently to keep out the frost. The experiment was basically divisible into two main parts (*a*) the transfer of infestation from potato and iris to potato, mint sowthistle and daffodil, and (*b*) the transfer from potato and iris to various iris varieties. The general layout and results are summarized in Tables 4 and 5. It was intended that examinations should be

TABLE 4. *Transfer of* D. destructor *to potato, mint, sowthistle and daffodil*

Plant tested	Source of inoculum: Potato	Iris, Sarah Bernhardt	Iris, Mansfield	Iris, Czar Peter	Iris, Queen of the Blues	Iris, Queen of the Violets	Iris, Wedgewood	Control
Potato, Duke of York	+ + + ○	+ + + +	+ + + +	+ + + −	+ + + +	+ + − −	+ + + +	− − − −
Mentha arvensis	+ + + +	+	+	+	+	+	−	
Sonchus arvensis	− − − −	−	−	−	+	−	−	
Narcissus, King Alfred		−	−	−	−	−		

+ = infested. − = clean. ○ = plant died.

TABLE 5. *Transfer of* D. destructor *to iris*

Plant tested	Source of inoculum: Potato	Iris, Sarah Bernhardt	Control
Rembrandt (D)	+ + − −	+ + + +	−
White Excelsior (D)	+ + + +	+ + + +	−
Wedgewood (D)	+ + + +	+ + + +*	−
La Nuit (S)	+ + ○ −	+ + ○ ○	−
Giant (S)	+ + − −	+ + + −	−
King of the Blues (S)	+ + + +	+ + ○ −	−
L'Innocence (S)	+ + − ○	+ + − −	−
Le Megol (S)	+ + ○ ○	+ + ○ ○	−
Mont Blanc (E)	+ + + +	− − − −	−

D = Dutch. S = Spanish. E = English. + = infested. − = clean. ○ = plant died.
* Wedgewood inoculum.

carried out on four separate occasions so the experiment was set up in a fourfold replication as far as possible. Control, uninoculated pots were planted whenever specimens allowed, as additional checks.

Examinations

The main examinations were carried out 3 and 5 months from the start of the experiment, i.e. 19 January 1949 and 14 March 1949, whilst the last examination took place when all the foliage had died down, the dates being 20 April 1949 for the potato pots and 1 June 1949 for the iris and daffodil pots. The final examination of the mint and sowthistle pots was on 2 September 1949.

The results of the examinations considered under the different hosts tested gave the following picture.

Potato. In twenty-four of the thirty-two pots of potato, infested tubers were found. In seven, including the four controls, no infestation had occurred and in one pot the plant had succumbed to the eelworm attack for rotten tubers were found. On the infested plants lesions were found on the old tubers, on the new tubers and on the underground parts of the haulm (Pl. 1, A, B and C). Specimens of *Ditylenchus destructor* were dissected out from all these lesions. On the tubers attack was generally through lenticels though in a few cases entry had occurred at an 'eyebrow'. Where heavy attack had taken place most of the tuber had rotted away.

Iris. There were seventy-six pots containing iris bulbs in this part of the experiment. Infestation had taken place in forty-nine of these; in nine pots the plants had died due in each case to the severity of the eelworm attack. Eighteen pots, including the eight controls, contained clean, healthy bulbs. Infestation had started in some instances from attack at the tips of the outer scales or on their surface, resulting in rotten areas, the worms being found mostly in the advancing edges of this rot. Infestation, showing as rot and discoloration of the tissues, was commonest, however, at the bulb base between the points of root emergence. The root system was usually poor, many of the roots were rotten and contained specimens of *D. destructor* in all stages of development. The Spanish varieties were attacked most heavily, resulting in some cases in complete rot of the bulb. During the final examination of the pots containing iris it was noticed that there were rather more clean bulbs than at the earlier examinations. It may have been that infestation had not taken place in these cases but it is also possible that the warm conditions prevailing in the greenhouse during May and June were responsible for some dying out of light infestations. Other observations have indicated that these nematodes thrive best under cool, moist conditions. The infestation in these bulbs was of a light nature and was seen as discoloured streaks on the tunic and as dried rotten tissue at the bulb base.

Mint. Transfer of the infestation from both potato and iris was successful in all instances with the production of characteristic dark brown lesions, containing numerous nematodes, in the cortical tissue of the rhizome internodes. The symptoms

shown by infestation from the two sources were indistinguishable from one another (Pl. 1, D and E).

Sowthistle. In the early examinations of the plants pre-adult specimens of *Ditylenchus destructor* were dissected out from between and within the tissues of the outer leaves of the dormant buds. It was thought that the attempt to set up infestation in sowthistle had been a failure, for lesions containing eelworms could not be found on any of the rhizomes. However, three pots had been left and when examined on 2 September 1949, one contained rhizomes bearing lesions from which several larvae of *D. destructor* were obtained by dissection. This particular pot had been inoculated with material from iris var. Queen of the Blues.

Daffodil. No symptoms of disease in the form of leaf 'spikkels' appeared and on lifting the bulbs later, no eelworms could be found in any of them.

Pathology

From the examinations made on the various occasions it is possible to build up a picture of the attack by *Ditylenchus destructor* on bulbous iris, when bulbs are planted in infested soil. Attack can and often does originate at the nose of the bulb and if it develops rapidly may cause death, though this is usually helped by the secondary invasion of fungi, bacteria and mites. In the foregoing experiments there was also attack on the surface of the outer bulb scale though this type of attack may be rather unusual. It must be remembered, however, that for the purpose of the experiment the protective tunics had been stripped from the bulbs. Where the roots emerge around the edge of the basal plate the tissue is loose and it seems quite easy for the eelworms to penetrate the bulb here. Invasion is commonest in this position and eelworms, having penetrated the basal plate, further invade the bulb scales. Invasion of the roots, which seems to be a hitherto unobserved type of damage, also takes place from the basal region. It usually causes grey-black rot in the proximal inch or two, the distal end remaining free; occasionally, though, invasion takes place direct from the soil, for isolated lesions are also found on the roots. When there is a heavy attack at the base of a bulb the root system may be very poor; the effect of this injury being seen in the leaves which are poorly developed and whose tips become yellow.

Once an attack has started the subsequent development of the plant depends partly on varietal susceptibility and partly on the severity of the invasion. In these experiments the Spanish varieties appeared to be the most susceptible and many plants succumbed to attack. The only English variety of iris, Mont Blanc, tested in the experiment, suffered considerable damage and in all four cases most of the bulb rotted away. This evidence and the presence of the heavy infestation in the English iris bulbs bought for the experiment indicates the very susceptible nature of this variety also. The Dutch varieties do not seem to be quite so susceptible, for none of the bulbs rotted away. In the early stages of growth of the Dutch varieties there was considerable rot in the outer scales of the mother bulbs, but the plants remained

healthy in appearance though the daughter bulbs produced by the three varieties tested were almost all infested. In general, a heavy infestation of a bulb usually causes its death but with a mild one the bulb continues to live and produce daughter bulbs.

The daughter bulbs in the iris arise from buds in the axils of the bulb scale of the parent bulb. The bases of the foliage leaves, after the season's growth, become part of the tunic of the daughter bulbs. When these leaf-bases have been infested the lesions show as dark streaks on the dry tunic and eelworms can usually be dissected from these areas when examined soon after lifting. After invasion of the bulb base the nematodes gradually travel into the bulb scales causing damage which is at first localized in streaks but which soon becomes more generalized. These lesions begin as dirty cream discolorations but develop to grey-brown and finally blackish rotten areas with the gradual destruction of the tissues (Pl. 2 A and B). Closer examination of such a lesion shows that it can be considered as divisible into three main zones bounded by undamaged living cells. In the outer zone, the advancing edge of attack, the eelworms are actively feeding on living cells and penetrating between them. The second zone in which the discoloration begins, becomes darker till the centre of the lesion, the third zone, is reached, where most of the cells are now dead. The discoloration is probably due to changes closely bound up with cell death and perhaps, also to the result of invasion by other organisms. The same sort of picture is seen in a potato tuber where most of the eelworms will be found amongst the cells at the periphery of a lesion, bordering on uninvaded tissue. This region appears glistening white, for there is an increase of intercellular air spaces and it is on the cells of this region that the eelworms feed.

Hastings (1949) has now shown a somewhat similar state of affairs caused by *D. dipsaci* in narcissus bulbs, for he has reported finding eelworms in the white scales as well as in rotting brown ones. In the English iris, particularly var. White Excelsior, the necrotic streaks are bright orange, though unfortunately this does not seem to be a specific indication of nematode attack but is a general host reaction to damage. Invasion also frequently occurs into the base of the flowering shoot where the damage shows as black streaks or flecks on the shoot up to ground level. As the season proceeds the foliage dies down, the axillary buds develop into the daughter bulbs and as their basal plates are intimately connected with that of the parent bulb it often happens that they become infested at an early stage and in some cases invasion reaches into the scales as well. Not all the daughter bulbs, however, of necessity become infested.

The biology of *Ditylenchus destructor*

In erecting *Ditylenchus destructor* as a new species Thorne did not discuss its biological relationships. A comparison, however, of the biology of the tuber nematode in iris and potato with that of the stem eelworm, *D. dipsaci*, in teasel or narcissus reveals certain points which help in the further differentiation of the two species.

Foremost amongst these features is the fact that so far as we know, *D. destructor* is confined to underground portions of the plant. In iris the parasite is found in the bulbs, in the roots and in the base of the flower stalk below ground level. In *Mentha arvensis* and in *Sonchus arvensis* it occurs in lesions on the rhizomes. In potato it is found principally in the tubers but also in lesions on the underground parts of the haulm (Pl. 1 A), a point which seems to have been overlooked in the past. The only exception to this generalization is the position the eelworms occupy in the dandelion, *Taraxacum officinale* Weber, where they are found between the leaves of the dormant rosette (Thorne, 1945; and in litt.). Here the eelworms are not only out of their usual underground habitat but, what is also very interesting, they are living outside the tissues of the plant too, in an apparently ectoparasitic relationship. Probably not too much stress should be laid on these points, for it may well be that the dandelion is only partially acceptable as a host, acting in a temporary capacity till another more suitable host becomes available.

There are no obvious symptoms shown by the above-ground parts of potato, mint and sowthistle when attacked by *Ditylenchus destructor*: plants known to be infested look quite healthy. In iris, however, varying degrees of ill-health are shown, such as yellow leaf tips or poor growth with flower failure and, in extreme cases, death. This difference in host response may possibly be connected with the habit of the plants involved. In potato, mint and sowthistle eelworm lesions do not occur in any vital parts and are, therefore, merely incidental, so that the existence of the host is not seriously imperilled by their presence. In iris, on the other hand, where the bulb is attacked, the whole plant suffers since the bulb is vital to the plant's life history and economy.

Another point of considerable biological interest and importance emerges in a consideration of the life cycle of the tuber nematode when compared with that of *D. dipsaci* in teasel or narcissus. Examination of populations of the stem eelworm in lightly infested, dormant narcissus bulbs during storage shows the eelworms in all stages of development, but if heavily infested bulbs are examined the so-called 'eelworm wool' is encountered. The same phenomenon is met with in seed heads of teasel, in onion bulbs and in the stems of *Vicia faba* attacked by the giant race of *Ditylenchus dipsaci*. In all cases the 'wool' appears to be formed when conditions in the host become inimical to the further life of the parasite. On the other hand, when populations of *D. destructor* are examined from potato tubers or iris bulbs, 'eelworm wool' never seems to occur. Even when the tuber or bulb is completely rotten there are no such quiescent, highly resistant, pre-adult larvae, the populations being mixed ones of eggs, larvae and adults. Seinhorst (1949) confirms the absence of pre-adult quiescent larvae in *D. destructor* infesting potato tubers in Holland and also shows that in addition to attacks by *D. destructor*, potato tubers in Holland are susceptible to attack from three different biological races of *D. dipsaci*.

REFERENCES

Anon. (1932). [*Ditylenchus dipsaci* on Iris sp.] In List of intercepted plant pests, 1930–1931. *U.S.D.A. Bur. Ent. Pl. Quarant.* p. 333.

Anon. (1935*a*). [*D. dipsaci* on *Iris tingitana* v. Wedgewood.] In Diseases of plants in the United States in 1933. *Plant Dis. Reptr Suppl.* **86**, 84.

Anon. (1935*b*). [*D. dipsaci* on *Iris histrioides*.] In List of intercepted plant pests, 1934. *U.S.D.A. Bur. Ent. Pl. Quarant.* p. 3.

Baker, A. D. (1948). Some notes on experimental infestation of potato tubers with the potato-rot nematode, *Ditylenchus destructor* Thorne, 1945. *Rep. ent. Soc. Ont.* (78), 32.

Goodey, J. B. (1950). Potato Tuber Eelworm and Iris Bulbs. *Nature, Lond.*, **165**, 495.

Goodey, T. (1929). The stem eelworm *Tylenchus dipsaci* (Kühn, 1858): observations on its attacks on potatoes and mangolds with a host list of plants parasitized by it. *J. Helminth.* **7**, 183.

Goodey, T. (1940). *The nematode parasites of plants catalogued under their Hosts.* Pp. 80. St Albans, England. Imperial Bureau of Agricultural Parasitology (Helminthology).

Goodey, T. & Goodey, J. B. (1949). Tuber-rot eelworm of potato and its weed hosts. *J. Helminth.* **23**, 89.

Hastings, R. J. (1935). Miscellaneous notes on some nematode diseases of plants in British Columbia. *Plant Dis. Reptr*, **19**, 26.

Hastings, R. J. (1949). Concerning the movement of the bulb eelworm *Ditylenchus dipsaci* (Kühn) Filipjev, in Narcissus bulbs. *Sci. Agric.* **29**, 345.

Hastings, R. J. & Bosher, J. E. (1936). Detection of *Anguillulina dipsaci* in bulbous iris. *Plant Dis. Reptr*, **20**, 300.

Hastings, R. J. & Bosher, J. E. (1938). The nature of bulb nematode (*Ditylenchus dipsaci*) populations in Supreme, Prince Albert and Imperator iris bulbs, and their control by thermal treatment. *Canad. J. Res.* **16**, 230.

Hastings, R. J., Bosher, J. E. & Newton, W. (1939). Bulb nematode control in iris by hot water. *Canad. J. Res.* **17**, 144.

Hodson, W. E. H. (1934). *Anguillulina dipsaci* (Kühn), as a pest of bulbous irises. *J. Helminth.* **12**, 33.

Hodson, W. E. H. (1935). Control of insect pests of bulbs: a survey of recent work. *Sci. Hort.* **3**, 192.

Hurst, R. H. (1948). The potato rot nematode, *Ditylenchus destructor* in P.E.I. *Proc. Canad. phytopath. Soc.* **14**, 17.

Newton, W., Hastings, R. J. & Bosher, J. E. (1937). The nematode disease of bulbous iris caused by *Ditylenchus dipsaci* (Kühn, 1858) Filipjev, 1936, and experiments on its control by bulb treatment. *Canad. J. Res.* **15**, 175.

Poeteren, van N. (1925). Verslag over de werkzaamkeden van den plantenziektenkundigen dienst in de jaar 1924. *Versl. Meded. PlZiekt. Dienst Wageningen*, **41**, 16.

Seinhorst, J. W. (1949). Stengelaaltjes en Knollenaaltjes bij Aardappelen. *Landbouwk. Tijdschr. Wageningen*, **61**, 638.

Staniland, L. N. (1935). Hot water treatment of bulbs and plants. *Rep. Seale-Hayne agric. Coll.* (11), 5.

Staniland, L. N. & Barber, D. R. (1937). *The efficiency of baths used for the hot-water treatment of narcissus bulbs.* London: H. M. Stationery Office.

Steiner, G. & Buhrer, E. M. (1933*a*). The bulbous irises as hosts of *Tylenchus dipsaci*, the bulb or stem nema. *Phytopathology*, **23**, 103.

Steiner, G. & Buhrer, E. M. (1933*b*). Observations on nematode diseases of plants. *Plant Dis. Reptr*, **17**, 33.

Thorne, G. (1945). *Ditylenchus destructor*, n.sp., the potato rot nematode, and *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936, the Teasel nematode (Nematoda: Tylenchidae). *Proc. Helminth. Soc. Wash.* **12**, 27.

Tschaen, E. (1932). [*D. dipsaci* on *Iris tingitana* and *I. filifolia*]. *Rev. Path. vég.* **19**, 34.

EXPLANATION OF PLATES 1 AND 2

Plate 1

A. Potato haulm showing dark lesions caused by *D. destructor*.
B. New potato var. Duke of York infested from iris var. Sarah Bernhardt.
C. L.S. of same.
D. *Mentha arvensis* rhizomes; black lesions caused by attack of *Ditylenchus destructor* from iris var. Queen of the Violets.
E. Portion of *Mentha arvensis* rhizome attacked by *Ditylenchus destructor* from potato; enlarged to show character and position of lesion.

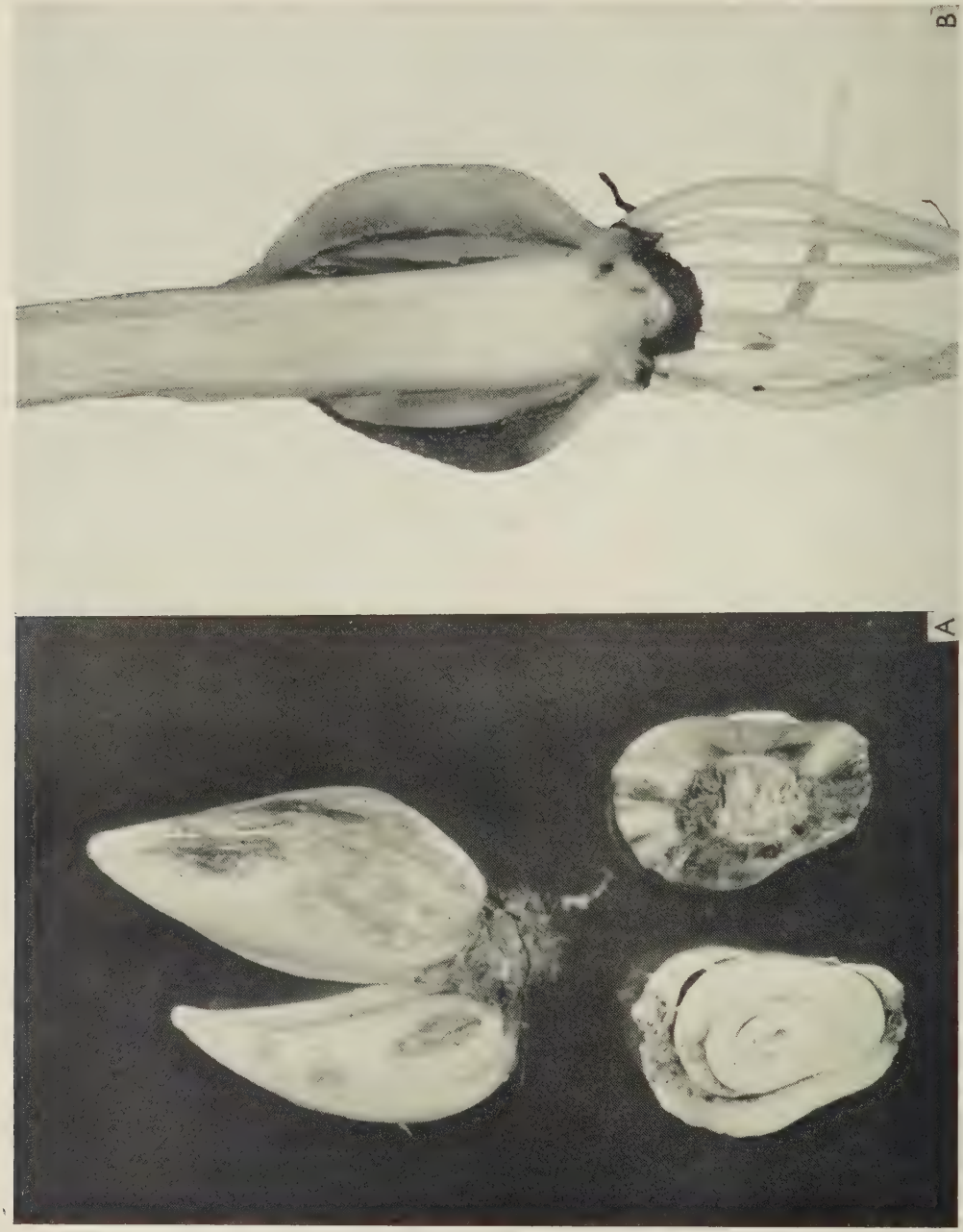
Plate 2

A. English iris var. Queen of the Violets infested with *D. destructor*; lateral view, basal view and T.S. of bulb.
B. Dutch iris var. Rembrandt infested with *D. destructor* from potato; L.S. of growing bulb.

(*Received 22 June* 1950)



GOODEY—*Potato and iris eelworm.*



GOODEY—*Potato and iris eelworm.*

INVESTIGATIONS ON THE SYSTEMIC INSECTICIDAL ACTION OF SODIUM FLUOROACETATE AND OF THREE PHOSPHORUS COMPOUNDS ON *APHIS FABAE* SCOP.

W. A. L. DAVID AND B. O. C. GARDINER

Agricultural Research Council, Unit of Insect Physiology, 34a, Storey's Way, Cambridge

(With 5 Text-figures)

The general insecticidal properties of *bis* (*bis*-dimethylamino phosphonous) anhydride (*anhydride*), *bis* (dimethylamino) fluorophosphine oxide (*oxide*), diethyl paranitro-phenyl phosphate (E 600), and sodium fluoroacetate (*acetate*) are described. All are toxic to aphids when infested plants are dipped in solutions. Although this is thought to be a contact action the possibility that the materials are first absorbed by the leaves and that the aphids are killed by imbibing toxic sap is not excluded. The order of decreasing toxicity by dipping is E 600 > acetate > anhydride = oxide. At the lowest concentration giving a complete kill of aphids, the anhydride is the most persistently effective of the four compounds. E 600 is the most phytotoxic compound.

When applied to the roots the order of decreasing toxicity is acetate > oxide > anhydride > E 600 in sand and soil, and acetate > oxide > anhydride = E 600 in culture solution. The anhydride and acetate are more persistent in the plants than the oxide and E 600. The margin of safety between insecticidal dosage and phytocidal dosage is appreciably larger with the acetate than with the other three compounds.

When equal dosages are administered to plants by the cut tap root technique the order of decreasing toxicity to aphids is seen to be acetate > oxide > E 600 > anhydride. All compounds, except possibly E 600, appear to be absorbed by the intact roots of the plants.

Systemic insecticidal action following application made to the leaves of the broad bean is easy to demonstrate with the acetate, demonstrable with difficulty with the anhydride and not at all with E 600 and the oxide.

Only the oxide is sufficiently volatile to kill insects by a fumigant effect either as the pure substance or after absorption into the plant. Plants treated with it give off a toxic vapour which may be collected by condensation and shown to be systemically active. The material concerned is presumed to be the unchanged oxide.

Sodium fluoroacetate is an extremely effective systemic insecticide whether applied to the leaves or the roots of the broad bean. It is apparently not phytotoxic at several times the concentration necessary for insecticidal action but may prove to be too generally toxic or persistent for practical use.

Introduction

A 'systemic' insecticide is one which is absorbed by a plant and translocated within it. The idea that trees and smaller plants could be freed from insects by substances acting systemically is many centuries old. The method has, however, never been much used because no satisfactory systemic insecticides have been discovered and a review of the literature shows in fact that only potassium cyanide and certain selenium compounds have ever been seriously considered.

No appreciable progress in the development of systemic insecticides seems to have been made until the work of Schrader and Kükenthal became generally known as a result of reports by Martin (1947), Martin & Shaw (1948) and Schrader (1948). Various workers have been able to substantiate many of the claims made by Schrader (Bennett & Martin, 1947; Bennett, Martin, Stringer & Woodcock, 1948; David, 1949; David & Kilby, 1949; Ripper, Greenslade & Hartley, 1950). The present paper gives further information concerning the systemic properties of three compounds developed by Schrader and Kükenthal and also about sodium fluoroacetate which has only recently been shown to possess potent systemic insecticidal activity (David, 1950*a*).

A systemic insecticide has certain advantages in comparison with a conventional insecticide. Since it poisons the sap, all insects feeding on the living tissues, on the surface or within the plant, are subjected to its action. Furthermore, since the insecticide is translocated within the plant, those parts missed in the application are also protected.

The ideal systemic insecticide would be persistent in the plant but of negligible toxicity to mammals. In the absence of this ideal, which is not attained by most of the familiar insecticides, it seems possible that non-persistent types may be developed for use on food crops and more persistent materials for use where continued protection is desired and toxic residues are unobjectionable.

Possible methods of applying systemic insecticides

Plants can be treated with systemic insecticides by three main methods:

(*a*) *Application to the roots.* The solid or liquid can be applied to the soil around the roots as is done with fertilizers. This method is only practicable with a cheap, relatively non-toxic insecticide. The method involves considerable losses of material in the soil and is likely to be particularly inefficient for deep-rooted plants and when the surface soil is dry.

(*b*) *Application to the foliage.* It is known that plant hormones and nutrients can be absorbed by the aerial parts of plants and this would seem to be the most economical and practicable way to get systemic insecticides absorbed. Besides entering through the leaves any insecticide which falls to the ground may be taken up by the roots.

(*c*) *Injection into the plant.* This method is only economical for trees or valuable plants. Both liquids and solids may be employed and little material is wasted. It has been shown (Roach, 1938; Roach & Roberts, 1945) that when using a solution the whole plant above the point of injection can be treated from a single injection hole if this is located correctly. The disadvantage of the liquid method is that large volumes of liquid are required. The solid method is convenient in so far as the bulk of material involved is quite small, but to ensure even distribution many holes have to be bored in the trunk and this damages the tree appreciably (Roach & Roberts, 1945).

Methods and equipment

1. *The greenhouse*

The tests were carried out in a greenhouse where the temperature, humidity, and the duration and intensity of light varied considerably. The worst extremes were avoided by adjusting the heating, windows, and blinds. In the winter the length of 'day' was increased to 16 hr. by fluorescent lighting giving about 3000 m.c. on the plants. The heating was supplemented by electric window warmers which kept the temperature on the plant benches above 15 C., and usually between 17 23 C.

To prevent diffusion of toxic vapours from treated culture solutions or potted plants, the greenhouse benches were covered with galvanized iron sheeting and a glass sash window was built along the front to meet the roof. The lower part of the window was usually kept half open and ventilation assisted by two extractor fans in the outside windows of the greenhouse.

2. *The test insects and plants*

Aphis fabae Scop., on broad beans, *Vicia faba* L., were used for the experiments. The beans were grown in individual 'Whalehide' pots $3\frac{1}{2}$ in. in diam., either in John Innes pasteurized potting compost or in washed silver sand watered once a week with a nutrient solution.

Aphids were obtained either from the 'production line' arrangement described by Kennedy & Booth (1950) or from stocks maintained on uncaged potted beans. When the experimental bean plants were about 4 in. high, they were infested by small pieces of aphid-bearing shoots from the stock plants.

During the tests the individual plant pots were isolated by being placed in Petri dishes surrounded by water held in flower-pot saucers.

Materials tested

Four highly purified compounds have been used for the tests. Two of these, *bis* (*bis*-dimethylamino phosphonous) anhydride (I) and *bis* (dimethylamino) fluorophosphine oxide (II) were recognized as powerful systemic insecticides by Schrader (Martin & Shaw, 1948; Schrader, 1948). The third compound, diethyl p-nitrophenyl phosphate (III) was also prepared and tested by Schrader. He considered that it was chiefly useful as a contact insecticide but possessed of some systemic activity.

$[(CH_3)_2N]_2\,PO.O.PO\,[(CH_3)_2N]_2$ (I)

$[(CH_3)_2N]_2\,POF$ (II)

$(C_2H_5O)_2\,PO.O.C_6H_4NO_2$ (III)

$CH_2FCOONa$ (IV)

The fourth compound tested was sodium fluoroacetate (IV) (David, 1950*a*). This has been used as a rodent poison (Dieke & Richter, 1946; Schrader, 1948) but the insecticidal properties of the salts of fluoroacetic acid have not previously been recognized (Schrader, 1948). Sodium fluoroacetate occurs in the poisonous South

African plant gifblaar (*Dichapetalum cymosum*) and it therefore seemed possible that it might be tolerated by other plants.

In the text, compound (I) is referred to simply as the *anhydride*, compound (II) is called the *oxide* and compound (III) E 600. All these were prepared in a pure state by Dr B. A. Kilby (David & Kilby, 1949). Compound (IV) is called the *acetate* and was supplied by Dr B. C. Saunders (Saunders & Stacey, 1948). All the compounds are highly toxic to mammals.

Table 1. *Showing certain properties of the four compounds*

	Anhydride		Oxide		E 600		Acetate	
Appearance	Colourless, almost odourless liquids				Pale yellow liquid		White crystalline solid	
Density	1·09 25°(5)		1·1127 25°(6)		—		—	
Boiling-point ° C.	98°/0·002 mm.(2) 102°/0·003 mm. 106°/0·004 mm.		86°/15 mm.(6)		173°/1 mm.(7)		—	
Solubility in water	Very soluble		Very soluble		0·2 % approx.		Very soluble	
Toxicity (mg./kg.)	Oral $L.D._{100}$	Subcut. $L.D._{100}$	Oral L.D.	Subcut. L.D.	Oral L.D.	Subcut. L.D.	Oral $L.D._{50}$	Subcut. L.D.
Rat	18(5)	18(5)	—	—	—	—	0·2(3)–7·0(4)	—
Mouse	—	—	3–5(7)	1–2(7)	2(7)	—	—	—

(1) Coates (1949); (2) David & Kilby (1949); (3) Dieke & Richter (1946); (4) Elizewitz (1947); (5) Ripper *et al.* (1950); (6) Saunders & Stacey (1948); (7) Schrader (1948).

The $L.D._{50}$ figures for sodium fluoroacetate against rats illustrate the wide variation with different species and strains of rats.

It was surprising to find that algae grew in 10 % solutions of the anhydride in distilled water. For this reason it was stored undiluted. Algae were not seen in solutions of the other compounds.

Assessment of the effect of the materials on the insects

The aphid population on a plant was assessed as follows:

3 Normal	Thriving colonies with aphids of all stages settled and feeding, or a few adults surrounded by young.
2 Declining	Colonies with few young aphids, some dead aphids on and around the plant, and the remaining aphids often moving restlessly about the plant.
1 Single	Only scattered individual aphids occurred and there were no young. Around the plant there were many dead aphids.
0 Nil	No aphids on the plant.

Besides the immediate toxic effect the persistence of the insecticide was investigated. Attempts were made to reinfest treated plants 2, 5 and 10 days after the

beginning of the experiment. The toxic condition shown by the plant at the time of reinfestation was then described as:

High	All the aphids dead within 2 days of reinfestation.
Moderate	All the aphids dead within 5 days.
Low	Some decline in population but an incomplete kill of aphids within 5 days.
Nil	No decline in population observed.

All the treatments were carried out in duplicate or triplicate and the results of all experiments have been confirmed.

Assessment of the effect of the materials on the plants

Phytotoxicity was assessed on the plants as follows:

Normal	Not different from controls.
Trace	Slight marginal leaf scorch or scattered spotting.
Moderate	Large areas of the leaves scorched and some entirely destroyed.
High	Plant almost entirely killed though the small crown leaves may be still green.

When working with broad beans it should be noted that the leaves may show areas of brown which are not due to systemic insecticides. These may arise from a variety of causes, including the fungus disease 'chocolate spot'.

Results

Dipping tests

When infested bean leaves were dipped into solutions of the insecticides the aphids were killed. It is not known whether the insecticides acted directly as contact poisons or fumigants or indirectly, after being absorbed by the leaves, as stomach poisons. This matter is being investigated.

The plants were dipped into solutions of the insecticides at 20° C. containing 0·1 % 'Teepol' as a wetter. Afterwards the plants were laid on their sides to dry, as a precaution against any draining of the solution down to the roots.

Sometimes only the lowest leaf pair was dipped. In preparation for this method the top of the plant was cut off 24 hr. before the test and all the aphids were allowed to transfer to the lowest leaf pair. The remaining leaf pair was then dipped in the insecticide in a Petri dish taking care not to immerse the cut stem. Any aphids on the undipped part of the plant were brushed off.

The persistence of the toxic effects was also investigated by reinfesting the plants as they became free of aphids. Control plants remained infested throughout the experiment.

The dipping tests were carried out on different dates as the four compounds did not become available simultaneously. Assuming that this did not affect the relative toxicities, it may be concluded that E 600 is most toxic to aphids and plants. Its high toxicity may be associated with its solubility in lipoids and therefore greater ability to penetrate the insect cuticle than the highly water-soluble anhydride,

Table 2. *Dipping tests with four insecticides*

Material	Concentration (%)	Aphids. First day on which Declining	Isolated	None	Toxic condition of plant: Last day Moderate	Low	First day Nil	Leaf damage level on 10th day
Anhydride	0·10	—	1	2	2	5	10	N.
	0·05	1	2	3	2	5	10	N.
	0·025	1	2	—	—	—	—	N.
	0·0125	2	—	—	—	—	—	N.
Oxide	0·10	—	1	3	—	—	2	T.
	0·05	—	1	3	—	—	2	N.
	0·025	1	3	—	—	—	—	N.
	0·01	—	—	—	—	—	—	N.
E 600	0·005	—	—	1	—	—	2	T.
	0·001	—	—	1	—	—	2	N.
	0·0005	1	2	5	—	—	2	N.
	0·0001	—	—	—	—	—	2	N.
Acetate	0·01	—	—	1	2	—	5	N.
	0·005	—	—	1	—	2	5	N.
	0·001	—	1	2	—	—	2	N.
	0·0005	2	—	—	—	—	—	N.

T. = trace. N. = nil.

oxide and acetate. The order of immediate toxicity is: E 600 > acetate > anhydride = oxide. At the lowest concentration giving a complete kill in each case the anhydride is the most persistent compound.

Systemic action following applications made to the roots

(*a*) *Plants growing in sand or soil.* For these experiments the beans were grown to a height of 6 in. in sand or potting compost in $3\frac{1}{2}$ in. diam. fibre pots. The moist sand and soil present weighed about 570 and 400 g. respectively.

The insecticides were dissolved in water and the required dose was made up to 20 c.c. Subsequently 20 c.c. of water was given to each plant daily unless the condition of the soil indicated that more was necessary.

Both the minimum quantities of the insecticides required to free the plants of aphids and the persistence of the toxic effects were investigated. The results of the experiments were assessed as already described. Other investigators have reported that the anhydride and particularly the oxide exert a direct fumigant effect when watered on to filter-papers under infested cabbage plants (Bennett *et al.* 1948). In our experiments, however, there was no evidence of a fumigant effect of any of the materials on the aphids on control plants standing among the treated plants.

The anhydride was tested, on plants growing in soil, at intervals during 1949 and early 1950. The results obtained showed that the effectiveness of a dose of 0·05 c.c. of anhydride increased between January and June of 1949 but that by January 1950 it had again decreased (Table 3). This observation may indicate merely a change in

the rate of uptake by the plants with temperature or insolation or some other seasonal change in the plants or aphids.

In order to compare the four compounds it is therefore necessary to test them simultaneously (Table 4A and B). These comparative tests were carried out in January but others which have shown the compounds to be more effective are also reported in Table 5. One longer period test was made with the anhydride especially to study persistence and this is shown in Table 6.

TABLE 3. *The seasonal change in the effectiveness of the anhydride*

Date of test	Temperature			Aphids. First day on which		
	Max.	Min.	Aver.	Declining	Isolated	None
12. i. 49	24	14	19·8	5	8	10
2. iii. 49	27	12·5	18·9	4	7	12
10. v. 49	27·5	14	20·0	4	6	7
6. vi. 49	30	13	20·9	2	5	6
24. i. 50	20	16	16·9	8	—	—

TABLE 4A. *Insecticide watered on plants in soil* (24 *January* 1950).

Material	Quantity applied	Aphids. First day on which			Toxic condition of plant	Leaf damage on
	(c.c.)	Declining	Isolated	None	Level/day	10th day
Anhydride	0·02	6	7	12	L./15	N.
	0·01	7	—	—	—	N.
	0·002	—	—	—	—	N.
	(c.c.)					
Oxide	0·02	—	3	4	H./10, M./15	T.
	0·01	3	4	5	M./5	N.
	(c.c.)					
E600	0·04 (40 c.c.)	6	10	—	—	N.
	0·02	7	—	—	—	N.
	0·01	—	—	—	—	N.
	(g.)					
Acetate	0·001	2	4	6	N./10	N.
	0·0002	5	—	—	—	N.
	0·0001	—	—	—	—	N.

H. = high. L. = low. M. = moderate. N. = nil.

In one case 40 c.c. of E600 solution was used because of the low solubility of this material in water.

Table 4B. *Insecticide watered on plants in sand* (23 *January* 1950)

Material	Quantity applied (c.c.)	Aphids. First day on which Declining	Isolated	None	Toxic condition of plant Level/day	Leaf damage on 10th day
Anhydride	0·02	3	—	5	H./5, M./10	M.
	0·01	4	7	9	L./10	T.
	0·002	—	—	—	—	T.
	(c.c.)					
Oxide	0·01	—	1	4	H./5, L./10	H.
	0·002	2	3	5	M./5, N./10	T.
	0·001	7	—	—	—	N.
	(c.c.)					
E600	0·02	5	—	—	—	T.
	0·01	—	—	—	—	N.
	0·002	—	—	—	—	N.
	(g.)					
Acetate	0·001	2	3	4	H./15	N.
	0·0002	3	4	7	L./10	N.
	0·0001	5	—	—	—	N.

H. = high. L. = low. M. = moderate. N. = nil. T. = trace.

Table 5. *Tests showing highest activity observed. Various dates*

Material, medium and date	Quantity applied	Aphids. First day on which Declining	Isolated	None	Toxic condition of plant Level/day	Leaf damage on 10th day
	(c.c.)					
Anhydride soil (6. vi. 49)	0·05	2	5	6	H./5, M./10	T.
	0·02	3	7	—	M./10	N.
	0·01	6	7	—	L./10, N./15	N.
	0·005	7	—	—	—	N.
	(c.c.)					
Oxide soil (25. iv. 49)	0·01	1	2	3	H./5, M./10	T.
	0·005	1	2	4	H./5, N./10	N.
	0·0025	1	2	4	L./5, N./10	N.
	0·001	2	3	—	L./2, N./10	N.
	(g.)					
Acetate sand (21. xi. 49)	0·005	1	2	3	H./10, M./>20	N.
	0·001	2	3	5	H./10, M./>20	N.
	0·0005	2	4	5	H./5, M./>20	N.
	0·0001	4	5	6	L./10	N.

H. = high. L. = low. M. = moderate. N. = nil. T. = trace.

TABLE 6. *A long period experiment with the anhydride added to soil* (10 *May* 1949)

Quantity applied (c.c.)	Aphids. First day on which			Toxic condition of plant				Leaf damage	
				Last day			First day	Day first	Level on 20th
	Declining	Isolated	None	High	Medium	Low	Nil	seen	day
0·10	3	5	7	25	45	—	—	15	M.
0·05	3	5	7	15	30	35	—	15	M.
0·025	5	7	9	—	30	35	45	—	N.
0·01	6	8	10	—	—	10	30	—	N.
0·005	—	—	—	—	—	—	—	—	N.
Control	—	—	—	—	—	—	—	—	N.

M. = medium. N. = nil

The foregoing experiments show that all the insecticides acted more quickly and at lower concentrations in sand than in soil. The relative order of effectiveness in both cases was

Acetate > oxide > anhydride > E 600.

The oxide acted more rapidly than the anhydride at the lowest effective doses tested.

E 600 did not give a complete kill of aphids and its persistence has not been investigated. On the basis of comparisons made at the lowest dosages giving complete kills it appeared that the acetate and anhydride were more persistent than the oxide. The oxide may be less persistent because it is known to be given off by the leaves, though several other explanations are possible.

The phytotoxic dose was not determined for E 600 because of its low systemic activity. That of the acetate was above the insecticidal range tested and there seemed to be a large margin between the insecticidal and phytocidal doses. The anhydride was less phytotoxic than the oxide, but it is doubtful whether its safety margin is much greater. It is difficult to give the precise phytotoxic concentrations as other factors can cause leaf-scorch almost indistinguishable from that due to the insecticides.

(*b*) *Plants in culture solutions.* The plants used for the tests were grown in compost or sand watered periodically with culture solution. The latter is preferable as it is easier to wash the roots in preparation for transferring the plants to the culture solutions. At the beginning of an experiment the plants were usually 4–8 in. high and weighed 8–15 g. The test solutions were contained in one-pound jam jars or half-pint milk bottles. It was not found practicable to aerate the large number of individual containers used but the solutions were changed and the bottles rinsed every 5 days.

The first experiments were planned to determine the concentrations of the insecticides effective against the aphids, the duration of toxicity, and the phytotoxicity of the compounds. In these first experiments the plants were either allowed to absorb the 100 c.c. originally given, in which case culture solution was supplied when the toxic solution was exhausted (Table 7), or the plants were transferred to

culture solution after 2–3 days when they had absorbed 15–30 c.c. of the toxic solutions (Table 8).

In a second series of experiments, designed to determine the lowest effective concentrations on prolonged treatment, the toxic solutions were renewed every 5 days, instead of being replaced by culture solution. The results of a comparative experiment carried out in this way are shown in Table 9.

In all cases the controls kept among the treated plants gave no evidence of a direct fumigant effect.

TABLE 7. *Plants allowed to absorb* 100 c.c. *of the toxic solutions followed by culture solution*

Substance and date	Concentration (%)	Aphids. First day on which Declining	Isolated	None	Toxic condition of plant: Last day High	Med.	Low	First day. Nil	Leaf damage: Day first seen	Level on 10th day
Anhydride	0·05	1	2	4	10	15	—	—	6	M.
(13. v. 49)	0·01	3	4	5	5	20	—	25	12	N.
	0·005	5	6	7	5	20	25	—	19	N.
	0·0025	5	6	8	5	—	10	20	—	N.
Oxide	0·05	—	—	1	10	—	—	—	2	D.
(12. iv. 49)	0·025	—	—	1	5	—	10	15	3	H.
	0·01	—	1	2	5	—	—	10	3	M.
	0·005	—	1	2	5	—	—	10	4	M.
	0·0025	1	—	2	5	—	—	10	4	T.

D. = declining. M. = medium. N. = nil. T. = trace.

From the experiments with the four compounds in culture solutions it may be seen that the relative order of toxicity was

Acetate > oxide > E 600 = anhydride.

The acetate and anhydride were more persistent than the oxide and E 600 at the lowest effective concentrations. And at these concentrations the oxide and E 600 were more phytotoxic than the anhydride and acetate. The margin of safety between the insecticidal and phytocidal concentrations for aphids on broad beans was greatest with the acetate.

Although in culture solution E 600 appeared to be about equal to the anhydride in systemic insecticidal activity, it was less active than the anhydride in soil and also much more phytotoxic in both cases. It would therefore be considerably less satisfactory as a systemic insecticide for root applications.

(*c*) *Concentrations of systemic insecticides in the plants necessary to kill the aphids.* Maximum values for the content of insecticide in the plants could perhaps be obtained from the foregoing experiments with insecticidal culture solutions but there are many sources of error. For example it is not known whether the roots selectively absorb or reject the insecticide in the culture solution and since the absorption is rather slow, appreciable quantities of the insecticide may be decomposed within the

TABLE 8. *Plants transferred to plain culture solution after absorbing 15–30 c.c. of toxic solutions*

Substance, volume and date	Concentration (%)	Aphids. First day on which			Toxic condition of plant				Leaf damage	
					Last day			First day.	Day first	Level on
		Declining	Isolated	None	High	Med.	Low	Nil	seen	10th day
Andyride	0·05	—	2	3	10	20	25	—	9	T.
15–20 c.c.	0·025	2	3	6	5	15	25	—	—	N.
(31. v. 49)	0·01	3	4	6	5	10	—	15	—	N.
	0·005	4	6	—	—	5	—	10	—	N.
	0·0025	6	—	—	—	—	5	10	—	N.
Oxide	0·05	—	1	2	5	—	—	10	3	H.
15–20 c.c.	0·025	—	1	2	2	5	—	10	4	H.
(31. v. 49)	0·01	—	1	2	2	—	5	10	4	M.
	0·005	1	2	3	2	—	—	5	6	T.
	0·0025	1	3	6	—	2	—	5	—	N.
E600	0·05	2	—	3	—	—	5	10	4	D.
25–30 c.c.	0·01	2	3	5	—	—	—	—	—	N.
(28. x. 49)	0·005	3	6	—	—	—	—	—	—	N.
Acetate	0·005	1	—	2	10	—	—	—	—	N.
25–30 c.c.	0·0005	1	2	3	10	—	—	—	—	N.
(21. xi. 49)	0·0001	2	4	5	—	10	—	—	—	N.
	0·00005	4	5	8	—	—	10	—	—	N.
	0·00001	—	—	—	—	—	—	—	—	N.

D. = declining. H. = high. M. = medium. N. = nil. T. = trace.

TABLE 9. *Comparative test in which fresh toxic solutions were given every 5 days* (16 *January* 1950)

Material	Concentration (%)	Aphids. First day on which			Leaf damage	
		Declining	Isolated	None	Day first seen	Level on 10th day
Anhydride	0·005	6	8	10	10	T.
	0·001	6	8	> 10	—	N.
	0·0005	> 10	—	—	—	N.
Oxide	0·005	1	2	3	8	T.
	0·001	3	6	> 10	—	N.
	0·0005	> 10	—	—	—	N.
E600	0·01	2	3	4	5	T.
	0·005	3	5	10	—	N.
	0·001	> 10	—	—	—	N.
Acetate	0·0005	—	2	3	—	N.
	0·0001	3	5	7	—	N.
	0·00005	5	6	7	—	N.
	0·00001	> 10	—	—	—	—

N. = nil. T. = trace.

plant or transpired from it. The errors can be reduced by arranging for the plant to absorb its dose rapidly and completely. This can be done by feeding 1 c.c. of the solution contained in a small specimen tube to the cut tap-root of the plant while the rest of the roots are in plain nutrient solution (Fig. 1). By this procedure the plant can be made to absorb its dose in a few hours on a sunny day.

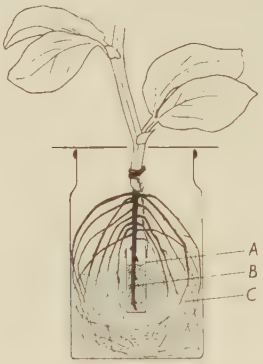


Fig. 1. The cut tap-root method of introducing known doses of systemic insecticides into plants quickly. *A*, capsule containing the insecticide; *B*, cut tap-root; *C*, jar containing the culture solution.

TABLE 10. *Bean plants given* 1 c.c. *lots of the toxic solutions by the cut tap-roots*

Material and date	Concentration (%)	Calculated conc. in plant (mg./kg.)	Day on which condition of aphid population became* 2	1	0	Toxic condition of plant (Level/day)	Day on which first damage to plant was noted
Anhydride	0·1	90	1st	2nd	3rd	M./10	11
(12. v. 49)	0·05	50	1st	2nd	3rd	M./5	11
	0·025	20	7th	—	—	L./5	—
	0·01	8	—	—	—	—	—
Oxide	0·1	62	—	1st	2nd	M./2	2
(30. iv. 49)	0·05	30	—	1st	2nd	—	3
	0·025	15	—	1st	2nd	—	4
	0·01	6	—	1st	4th	—	4
	0·01	6	1st	3rd	—	—	—
E600	0·05	28	1st	2nd	3rd	—	>5
(21. ii. 50)	0·01	5·5	—	—	—	—	>5
Acetate	0·01	9·0	—	—	1st	H./2 M./5	>10
(15. xi. 49)	0·002	1·2	1st	3rd	5th	—	>10
	0·001	0·9	1st	2nd	3rd	—	>10
	0·001	0·6	1st	7th	0	—	>10

H. = high. L. = low. M. = medium.

* 2 = declining colony; 1 = single scattered aphids; 0 = no aphids.

It may be seen from Table 10 that the order of toxicity of the four compounds

and the approximate concentration of insecticide expressed as mg./kg. of plant tissue found to give complete kills are:

Acetate 1 mg. > oxide 6 mg. > E600 30 mg. > anhydride 50 mg.

At these near-minimum effective concentrations only the anhydride shows any persistent toxicity. As usual the oxide is more phytotoxic than the anhydride and there is a larger margin of safety with the acetate.

(*d*) *The materials absorbed by intact roots.* When a plant is unpotted, especially from compost, many of the roots are damaged. It is known that fast green dye is absorbed by damaged roots but not by intact roots (Hitchcock & Zimmerman, 1935) and it was therefore of interest to determine whether the four insecticides penetrated into roots which were intact except for the fine root-hairs. Essentially the same procedure was adopted as in the experiments using the cut tap-root technique described in the previous section, except that in this case two to four undamaged roots were immersed in the small specimen tube of insecticide, while the rest of the roots were in culture solution. By this method the insecticides were absorbed more slowly but the insects were killed, except with E600. The latter compound was ineffective in dilute solution and damaged the roots at higher concentrations.

Absorption and translocation within the plant following application to the leaves

Except possibly in the case of trees the most convenient and economical method of using a systemic insecticide is to apply it to the foliage. Absorption will then have to take place mainly through the cuticle as it has been shown that the stomata are very difficult to wet and that water only enters the intercellular spaces when the leaf is completely immersed and kept in darkness (Sen & Blackman, 1933). However, the leaf cuticle is known to be permeable to certain inorganic salts and to herbicides (Fogg, 1948) and so may be expected to be permeable to insecticides. Translocation out of the leaf, the other requirement for systemic action, is also known to occur with nutrient salts and dyes and the ensuing distribution in the plant has been mapped in several species (Roach, 1938; Hill & Roach, 1940).

The first report of systemic action following applications made to the leaves in the literature is that of Fulton & Mason (1937) concerning rotenone. Their observation does not seem to have been followed up or confirmed. The next reports concern the compounds investigated by Schrader and Kükenthal (Martin & Shaw, 1948; Schrader, 1948) and many of their claims have been confirmed by other workers (Ripper, Greenslade & Lickerish, 1949; Ripper *et al.* 1950; David, 1950*b*). It has subsequently been shown that sodium fluoroacetate is also translocated following application to the leaves (David, 1950*a*).

Translocation of a systemic insecticide is most likely to occur when the plant is in an active condition and in the direction in which nutrients or reserves are being moved.

The experiments described later in the paper were all planned to investigate

translocation from mature leaves to the younger parts of actively growing bean plants. Systemic action of the anhydride against aphids could not be demonstrated when a lower leaf of the experimental plant was dipped one or more times in the solution of anhydride containing a wetter or when the tip three-quarters of the leaf was immersed continuously in the solution for several days. Only when the same solution was dripped over a leaf for several days or when the whole of the plant, except the crown, was dipped in an 0·2 % v./v. solution of the anhydride was some evidence of an effect on the aphids obtained. With the acetate on the other hand systemic action occurred after dipping a single pair of leaves. The procedure finally adopted was to dip a pair of leaves in 20 c.c. of the solutions incorporating 0·1 % 'Teepol' as a wetter. The plants were allowed to dry in a horizontal position so that none of the solution ran down to the roots nor dripped on to other untreated leaves. A filter-paper collar was then placed round the main stem above the treated leaves to prevent the living aphids walking on to the treated leaf and to collect the dead aphids. The only evidence of systemic action was obtained with the acetate. As this does not give rise to toxic vapour, as will be shown later, the aphids must have been killed by the translocated insecticide.

Table 11. *Systemic action after dipping one pair of leaves* (31 *January* 1950)

		Day after first dipping											
		1		2		4		5		6		7	
Insecticide	Untreated portion of plant examined	I	D	I	D	I	D	I	D	I	D	I	D
Anhydride	All above dipped leaves	3	0	3	3	3	7	3	10	3	11	3	14
Oxide	All above dipped leaves	3	0	3	3	3	5	3	5	3	7	3	10
E600	All above dipped leaves	3	0	3	1	3	5	3	7	3	7	3	8
Acetate	Crown and leaf pair above dipped	3		1		0		0		0		0	
			14		76		313		371		413		413
	Leaf pair opposite—above that dipped	3		3		2		1		0		0	

I = population level. D = number dead on the untreated portion of plant.

It seemed possible that absorption would occur most easily when the dilute solutions were allowed to dry and concentrate on the leaves than when the leaf was permanently immersed in the dilute solution. In the former case, however, the quantity of insecticide left on the leaves was small. In an attempt to combine the favourable aspects of these two methods, i.e. a high concentration and a larger quantity of material, 50 c.c. of the insecticidal solution were dripped over a leaf after which it was allowed to dry and the process was repeated several times daily. The apparatus is shown in Fig. 2 which needs no explanation except to say that a notch was cut in the funnel to take the petiole of the leaf being treated.

E600 was tested at 0·01 % and 0·1 % v./v. At both concentrations the treated

leaflets were quickly damaged and there was no evidence of systemic action. The aphids on the underside of the treated leaflets were killed.

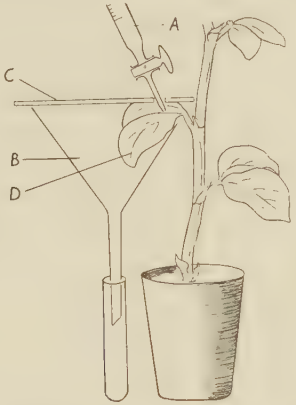


Fig. 2. The method used for dripping systemic insecticides over a leaf. *A*, burette containing the insecticide; *B*, funnel with a slot in the side closed by the perspex plate *C*; *D*, the treated leaf.

The oxide was tested at 0·05 % v./v. and gave no evidence of systemic action within 6 days, by which time the treated leaflet was damaged.

Four experiments were made with the anhydride at 0·1 % v./v. In one of these all the aphids on the plant on leaves above and below the treated leaflet were killed, but in the three others there was no evidence of systemic action.

Sodium fluoroacetate was tested at 0·01 % v./v. and invariably killed all aphids on the plants within about 5 days.

The course of one successful experiment with the anhydride, one with the acetate and a control are shown in Table 12.

It is interesting that the anhydride is very ineffective as a systemic insecticide in the bean plant. This is probably due to some property of the bean, as it is effective in other plants. The failure of the oxide may have quite another explanation. It has a higher vapour pressure than the anhydride and plants treated with it are known to give off a toxic vapour presumed to be the oxide (see p. 108). In this way the oxide may be lost during translocation so that a toxic concentration is never built up in untreated leaves.

The fumigant action of the insecticides either directly or from treated plants

A simple method of determining whether solutions of the insecticide give off toxic vapours is to enclose infested bean leaves under glass crystallizing dishes together with 4 cm. diam. filter-papers carrying the insecticide. The arrangement adopted is shown in Fig. 3. The volume of the enclosure was about 500 c.c. The

petiole of the leaf passed through a slot in the perspex floor. The results of experiments carried out in this way are given in Table 13.

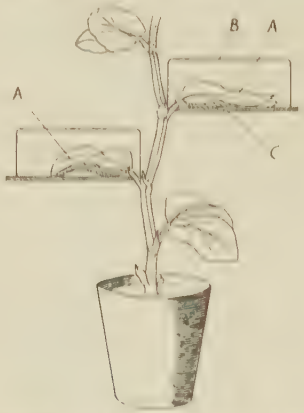


Fig. 3. The arrangement for testing the fumigant effect of the insecticides. *A*, leaf infested with aphids; *B*, crystallizing dish; *C*, perspex plate with a slot for the petiole of *A*. The retort-stand supports for *C* are not shown.

TABLE 12. *Translocation in the broad bean after dripping* 50 c.c. *of solution over a leaf* (5 *December* 1949).

Leaf no.	Position relative to treated leaf	Material and plant	Levels of populations on leaves on days									
			1	2	3	4	5	6	7	8	9	10
1	Above (tip)	Anhydride	3	3	3	3	3	3	2	2	0	0
2	2nd above	0·1 %	3	3	3	3	3	3	2	1	1	0
3	1st above (opposite)		3	3	3	3	3	3	2	1	0	–
4	Treated leaf		3	1	1	0	0	0	0	0	0	0
5	1st below (opposite)		3	3	3	3	3	3	3	2	0	0
1	Above (tip)	Acetate	3	3	3	2	0	0	–	–	–	–
2	3rd above (opposite)	0·01 %	3	3	3	3	2	0	–	–	–	–
3	2nd above		3	3	2	2	1	0	–	–	–	–
4	1st above (opposite)		3	3	2	1	0	0	–	–	–	–
5	Treated leaf		3	0	0	0	0	0	–	–	–	–
6	1st below (opposite)		3	3	3	2	1	0	–	–	–	–
1–5	All positions	Control	3	3	3	3	3	3	3	3	3	3

TABLE 13. *The fumigant action of the four insecticides*

Material	Mg. in enclosure	Population levels on days after treatment								
		0	1	2	3	4	5	6	7	8
Anhydride	10·0	3	3	3	3	3	3	3	3	3
Oxide	10·0	3	1	0	0	0	0	0	0	0
	0·05	3	1	0	0	0	0	0	0	0
E600	0·1	3	3	3	3	3	3	3	–	–
Acetate	0·1	3	3	3	3	3	3	3	3	3
Control	0	3	3	3	3	3	3	3	3	3

It may be seen from Table 13 that at the concentrations tested only the oxide exerted a detectable toxic action as a vapour. However, it seemed possible that within the plant the other compounds might give rise to volatile toxic substances. Using the technique just described, leaves from plants which had been standing in 0·05 % solutions of the four compounds were enclosed with infested leaves. Only the leaves from the plant treated with the oxide gave off a toxic vapour. This observation was confirmed in several experiments. The weight of treated leaves used in each case was about 0·5 g. In the case of the leaves treated with oxide, all the aphids on the untreated leaves were killed in 1 2 days. Bennett *et al.* (1948) have noted that leaves treated with the oxide exert a strong fumigant action on the brassy willow beetle *Phyllodecta vitellinae* L.

The possibility that the oxide had been converted into a still more toxic compound by the plant was investigated by comparing the toxic effect produced by leaves from a plant treated with a known quantity of the oxide by the cut tap-root technique and that of the oxide applied directly to disks of filter-paper. Ignoring all losses which may occur and the possibility of uneven distribution within the plant, the maximum quantity of oxide in a known weight of the treated plant can be calculated. The results of one such experiment are given in Table 14. It may be seen that there is no evidence of the synthesis of a compound which is appreciably more toxic than the oxide.

TABLE 14. *The fumigant action of leaves and filter-papers treated with the oxide*

Infested leaf no.	Source of oxide	Calc. wt. of oxide (mg.)	Levels of populations on days				Aphids killed (%)
			0	1	2	3	
1	0·65 g. leaf	0·16	3	1	1	1	95
2	0·25 g. leaf	0·06	3	3	2	2	75
3	0·07 g. leaf	0·02	3	3	3	3	<5
4	0·1 c.c. of 0·05 % sol.	0·05	3	1	0	0	100
5	0·1 c.c. of 0·025 % sol.	0·025	3	1	0	0	100
6	None	Nil	3	3	3	3	<5

In the experiments just described the oxide vapour might be killing the aphids feeding on the leaves directly by its fumigant action or indirectly after it has been absorbed by the leaves and has made the sap toxic. To test these alternatives a Petri dish was placed over an infested leaf, so that half of it was enclosed with another leaf from a plant treated with the oxide, while the rest of the infested leaf was outside the enclosure. The aphids within the enclosure were killed while those 1–5 mm. outside it were unaffected (Table 15). Had the action of the oxide been dependent upon previous absorption by the leaf, it would seem reasonable to suppose that it would have been translocated this short distance within the leaf.

Table 15. *Absorption of toxic vapour by a leaf*

	Levels of populations on days					
Position of aphids ...	0	1	2	3	4	5
Enclosed with treated leaf	3	0	0	0	0	0
Outside enclosure	3	3	3	3	3	3

It is evident that plants treated with the oxide give off a toxic vapour. This may be further confirmed by enclosing the aerial parts of an intact plant growing in a solution of the oxide (0·05 %) with those of one growing in culture solution (Fig. 4). The aphids on the plant in the insecticide are killed in 2 days and those on the plant in culture solution in 4 days. If the top of the untreated plant is kept outside the enclosure the aphids on it are unaffected after 10 days. This observation again confirms that oxide vapour is not absorbed and translocated within the untreated plant at any rate in toxic concentrations.

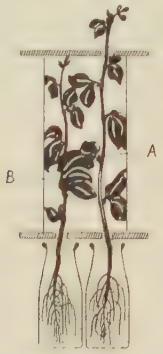


Fig. 4.

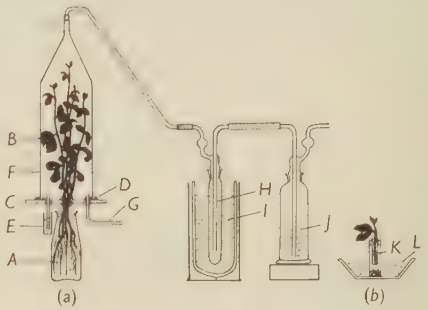


Fig. 5.

Fig. 4. A control plant *A* is enclosed with a plant absorbing a solution of the oxide *B*.

Fig. 5*a*. Collection of material transpired by plants treated with the oxide. *A*, bottle containing the insecticide; *B*, group of treated plants; *C*, perspex plate slotted and fitted with a slide and two rings filled with petrolatum *D* and the small manometer *E*. The cylinder *F* drops between the rings *D* to form a seal. Dry compressed air is supplied via *G*. The condensate collects in *H* which is cooled in *I*. *J*, bubbler to check the air flow. Vacuum is applied to *J* so that there is a barely perceptible negative pressure balance in cylinder *F*.

Fig. 5*b*. The arrangement for testing the toxicity of the condensate. The condensate is placed in *K* and the dead aphids collect in the water-barrier *L*.

The toxic material transpired by a plant treated with the oxide has been collected by the following procedure. The aerial parts of three or four plants, placed together in 200 c.c. of an 0·05 % solution of the oxide the day previously, were enclosed in a cylinder while the roots remained outside in the solution. The cylinder was kept in a bright light and dry air was passed through it. After passing over the plants the

air stream was carried through a tube cooled to about −70° C. to condense out the water and toxic material (Fig. 5). The solution obtained was tested for toxicity to aphids by placing in it the infested tops of bean plants severed from the parent plants. The aphids on the bean-top placed in the condensate were killed while those on the bean-top in water increased in number from 168 to 200 during the experiment. In other experiments with the anhydride, E600 and the acetate, the condensate obtained and tested by the above procedure did not kill the aphids.

TABLE 16. *The systemic action of material transpired by bean plants treated with the oxide*

Material	Total no. of aphids present at start	Percentage fallen off bean-tops on days				
		0	1	2	3	4
Water	168	1	9	11	12	12
Condensate	160	0	52	95	99	100

DISCUSSION

It is evident from the experiments described that the four compounds concerned in the tests are powerful insecticides, but it is not always easy to determine whether a material is acting chiefly as a fumigant, stomach poison or contact insecticide. For example, when aphid-infested leaves are dipped in the solutions besides a contact or fumigant effect the insecticide may also penetrate into the leaves and act as a stomach poison. Again where the insecticides are applied systemically both stomach poison and fumigant action may occur. These questions need further investigation but meanwhile it seems fairly certain that all the compounds can act systemically as stomach poisons although with the oxide fumigant action is almost certainly occurring simultaneously. There is no evidence from the experiments that any compound besides the oxide acts as a fumigant and in this respect we differ from Bennett *et al.* (1948) who have detected a fumigant effect with a commercial sample of the anhydride. E600 is much less soluble in water than the other compounds and perhaps for this reason does not seem to be very readily absorbed by the roots of plants and is not very effective as a systemic insecticide. It is highly toxic in dipping tests where it probably acts as a direct contact insecticide.

We should like to thank Dr B. A. Kilby, who has supplied the anhydride, oxide and E600, and Dr B. C. Saunders for supplying the acetate. All the materials were highly purified.

REFERENCES

BENNETT, S. H. & MARTIN, H. (1947). The qualitative examination of insecticidal properties —Progress report 1947. *Ann. rep. Long. Ashton Res. Sta., Bristol*, pp. 147.

BENNETT, S. H., MARTIN, H., STRINGER, A. & WOODCOCK, D. (1948). The qualitative examination of insecticidal properties—Progress report 1948. *Ann. rep. Long Ashton Res. Sta., Bristol*, pp. 138.

Coates, H. (1949). The chemistry of phosphorus insecticides. *Ann. appl. Biol.* **36**, 156.

David, W. A. L. (1949). Experiments with organo-phosphorus insecticides. *Rep. 2nd int. Congr. Plant Prot., London, July* 1949. (In the Press.)

David, W. A. L. (1950*a*). Sodium fluoroacetate as a systemic and contact insecticide. *Nature, Lond.*, **165**, 493.

David, W. A. L. (1950*b*). Insecticidal action of radioactive bis (*bis*-dimethylaminophosphonous) anhydride. *Nature, Lond.*, **166**, 72.

David, W. A. L. & Kilby, B. A. (1949). Preparation and insecticidal action of bis (*bis*-dimethylamino) phosphonous anhydride. *Nature, Lond.*, **164**, 522.

Dieke, S. H. & Richter, C. P. (1946). Comparative assays of rodenticides on wild Norway rats. I. Toxicity. *Publ. Hlth Rep.* **61** (19), 672.

Elizewitz, H. (1947). Epidemic control with D.D.T., A.N.T.U. and 1080. *Soap*, **235**, 127; **236**, 153.

Fogg, G. E. (1948). The penetration of 3:5-dinitro-*o*-cresol into leaves. *Ann. appl. Biol.* **35**, 315.

Fulton, R. A. & Mason, H. C. (1937). The translocation of derris constituents in bean plants. *J. agric. Res.* **55**, 903.

Hill, H. & Roach, W. A. (1940). Injection for the diagnosis of mineral deficiencies in the tomato, the potato, and the broad bean. *Ann. Bot.* n.s. **4**, 505.

Hitchcock, A. E. & Zimmerman, P. W. (1935). Absorption and movement of synthetic growth substances from soil as indicated by responses of aerial parts. *Contr. Boyce Thompson Inst.* **7**, 447.

Kennedy, J. S. & Booth, C. O. (1950). Method for mass rearing and investigating the host relations of *Aphis fabae* Scop. *Ann. appl. Biol.* **37**, 451.

Martin, H. (1947). Important new discoveries in plant protection. *The Grower*, **27** (17), 481.

Martin, H. & Shaw, H. (1948). Developments in methods and materials for the control of plant pests and diseases in Germany. B.I.O.S. Final Report no. 1095, 93 pp. London: H.M.S.O.

Ripper, W. E., Greenslade, R. M. & Lickerish, L. A. (1949). Combined chemical and biological control of insects by means of a systemic insecticide. *Nature, Lond.*, **163**, 787.

Ripper, W. E., Greenslade, R. M. & Hartley, G. S. (1950). A new systemic insecticide *bis* (*bis*-dimethylamino phosphonous) anhydride. *Bull. ent. Res.* **40**, 481.

Roach, W. A. (1938). Plant injection for diagnostic and curative purposes. *Imp. Bur. Hort. and Plant Crops, Tech. Comm.* no. 10.

Roach, W. A. & Roberts, W. O. (1945). Further work on plant injection for diagnostic and curative purposes. *Imp. Bur. Hort. and Plant Crops, Tech. Comm.* no. 16.

Saunders, B. C. & Stacey, G. J. (1948). Toxic fluorine compounds containing the C–F link. *J. chem. Soc.* 1773.

Schrader, G. (1948). The development of new insecticides. *B.I.O.S.* Final Report no. 714 (revised), 63 pp. London: H.M.S.O.

Sen, P. K. & Blackman, V. H. (1933). On the conditions leading to the injection of leaves submerged in water. *Ann. Bot.* **47**, 663.

(*Received* 8 *June* 1950)

THE INSECTICIDAL ACTIVITY OF PARATHION, ITS ISOMERS AND SOME RELATED COMPOUNDS

By D. WOODCOCK and A. STRINGER

Long Ashton Research Station, University of Bristol

(With 1 Text-figure)

Heat treatment of parathion at 140° C. and above resulted in isomerization and then thermal decomposition; the loss of toxicity to *Calandra granaria* being correlated with a reduction of the thiono-sulphur content. Similar results were obtained with O:O-dimethyl-(4-nitrophenyl)-thiophosphate. Paraoxon, O:S-diethyl-(4-nitrophenyl)-thiophosphate, and O:O-diethyl-S-(4-nitrophenyl)-thiophosphate, although all possessing considerable contact activity, were less insecticidal than parathion; O:O-dimethyl-(4-nitrophenyl)-thiophosphate, on the other hand, was considerably more effective than parathion. O:O-*bis*(2-chloroethyl)-O-(4-nitrophenyl)-thiophosphate was considerably less toxic than parathion and appeared to have a different mode of action. Compounds, S-ethyl-*bis*-(4-nitrophenyl)-thiophosphate, O-ethyl-*bis*-(4-nitrophenyl)-thiophosphate, and triethyl thiophosphate were ineffective as contact insecticides.

In the series of compounds examined, replacement of the group P = S by P = O or alteration in size of the groups attached to the central phosphorus atom caused a reduction of insecticidal activity.

Introduction

The value of O:O-diethyl-O-(4-nitrophenyl) thiophosphate (Schrader, 1947) as an insecticide is now well established, and its extreme potency as both a stomach and a contact insecticide has been confirmed (Bennett, S. H., Martin, H., Stringer, A. & Woodcock, D. 1948; Stringer, 1949).

Two factors which may influence the potency of parathion samples are: first, impurities incidental to manufacture and secondly, isomeric compounds produced by subsequent heat treatment. The former may be removed chemically or by distillation, but a prolonged fractionation has been shown to lead to rearrangement resulting in a loss of toxicity. This isomerization of the thiophosphates, first described by Emmett & Jones (1911), was investigated in some detail by Schrader (1947), who showed that the change (I)-(II) begins at a temperature of 130–140° C.

Technique

The test insects were adult grain weevils (*Calandra granaria* L.), and the usual precautions were taken to standardize the rearing and pretreatment of the insects and the method of testing. Batches of fifty *Calandra* adults were enclosed, by means of glass rings, on filter-papers which had been previously treated with insecticide (Stringer, 1949). The results were analysed by the usual statistical procedure, the regression of probit mortality on log dosage (Finney, 1947).

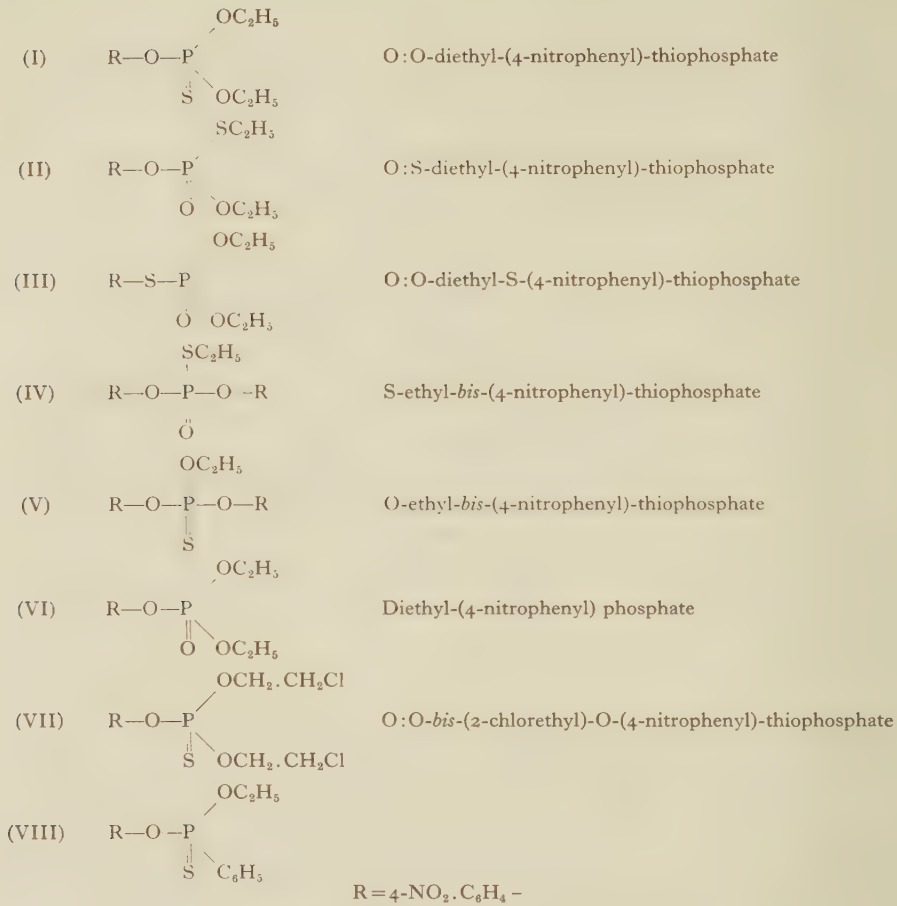


Fig. 1. Structural formulae.

MATERIALS

Commercial preparations of parathion are likely to contain, in addition to the main constituent, unreacted 4-nitrophenol, triethyl thiophosphate and ethyl-*bis*-(4-nitrophenyl)-thiophosphate. The phenol is known not to possess strong insecticidal properties but toxicity tests were made on the other two compounds. An attempted separation of crude parathion into its components by distillation *in vacuo* after washing out unreacted phenol gave unidentified fractions which contained variable amounts of sulphur easily oxidized by 55 % nitric acid. The latter reagent was suggested by Schrader (1947) for the estimation of sulphur present in the thiono-form (i.e. doubly linked to phosphorus in contrast to the mercapto-form in which it is present in the group $\equiv PSC_2H_5$).

The fractions subjected to bioassay were as follows: Triethyl thiophosphate. Sample no. R. 7323 supplied by Messrs Albright and Wilson Ltd., was a colourless liquid. (Found: ≡P=S, as triethyl thiophosphate, 109·3 %.)

Ethyl *bis*-(4-nitrophenyl)-thiophosphate. Albright and Wilson, sample no. R. 7395, was a very pale yellow crystalline solid, m.p. 126–128° C. (Found: ≡P=S as ethyl-*bis*-(4-nitrophenyl)-thiophosphate, 93·5, 93·2 %.)

Diethyl-(4-nitrophenyl)-thiophosphate. Purified by rapid distillation *in vacuo* and supplied by Messrs Albright and Wilson, sample no. R. 7401. (Found: thiono-sulphur, in terms of diethyl-(4-nitrophenyl)-thiophosphate, 80·7, 81·3 %.)

Parathion. Messrs Plant Protection Ltd., sample no. PP. 288 YX. (Found: thiono-sulphur as diethyl-(4–nitrophenyl)-thiophosphate, 104·5, 106·8 %.)

Fraction C. Recovered at 120–180° C./0·4 mm. from crude parathion, sample no. PP. 288 YX, in 29·0 % yield. (Found: thiono-sulphur as diethyl-(4-nitrophenyl)-thiophosphate, 50·9, 51·5 %.)

Fraction D. Recovered at 120–180 °C./0·4 mm. from crude parathion, sample no. PP. 288 YX, in 52·3 % yield. (Found: thiono-sulphur as diethyl-(4-nitrophenyl)-thiophosphate, 17·2, 17·5 %.)

Table 1. *Contact action of parathion*

	% kill at 24 hr.				% paralysis at 24 hr.	
Dosage mg./paper	R. 7401	PP. 288 YX	Fraction C	Fraction D	Fraction C	Fraction D
1·25	100	100	94	17	100	92
0·99	100	100	85	8	100	46
0·70	100	100	77	0	100	6
0·53	100	100	52	0	100	0
0·40	100	100	17	0	83	0
0·30	98	96	6	0	25	0
0·22	94	88	2	0	0	0
0·17	81	75	0	0	0	0

The results of toxicity tests against *C. granaria* are assembled in Table 1. Triethyl-thiophosphate and ethyl-*bis*-(4-nitrophenyl)-thiophosphate were non-toxic and provisional regression lines for the remaining samples gave the following estimated relative potencies:

Diethyl-(4-nitrophenyl)-thiophosphate	1·00	—	—
Parathion (PP. 288 YX)	0·83	1·00	—
Fraction C	0·17	0·21	1·00
Fraction D	0·05	0·06	0·31

The potency of the parathion sample relative to the purified sample is in agreement with expectations, but samples C and D show an unexpected loss of toxicity, though their relative potencies (1·00:0·31) are in good agreement with their thiono-sulphur content (51·2:17·3 %).

Heat treatment

Schrader (1947) has stated that isomerization of diethyl-(4-nitrophenyl)-thiophosphate begins at 130–150° C., and in view of this fact it was decided to investigate the nature of the change induced by heat treatment.

Samples were heated in a Pyrex flask under a short ground-jointed reflux condenser in a thermostatically controlled oil-bath. The temperatures given are those of the bath. For estimation of $\equiv P=S$ groups Schrader's method of oxidation by refluxing with 55 % nitric acid was used, the sulphuric acid being estimated gravimetrically.

The purest parathion sample (80 %) available at that time (late 1948) was heated for 24 hr. at 130–140° C. The liquid darkened in colour and became viscous; a highly crystalline substance was deposited on cooling. Trituration with ethyl alcohol gave a solid, m.p. 100–106° C., which recrystallized from methyl alcohol in colourless rectangular prisms, m.p. 111·0–111·5° C. (Found: C, 44·1; H, 3·3; N, 7·0 %; M.W. (Rast) 328: $C_{14}H_{13}O_7N_2PS$ requires C, 43·7; H, 3·4; N, 7·3 %; M.W. 384.) Hydrolysis by refluxing 1 hr. with 10 % aqueous sodium hydroxide resulted in a solution giving positive reactions for PO_4''' and S''. Acidification and extraction with ether gave an oil which was then acetylated. The acetate, crystallized from aqueous methyl alcohol in stout prisms, m.p. 75–77° C., was undepressed by an authentic specimen of 4-nitrophenyl acetate. Oxidation with 55 % nitric acid gave no sulphuric acid and therefore the $\equiv P=S$ linkage was considered to be absent. Treatment with fuming nitric acid at 0° C. gave a solid nitro-derivative on pouring on to ice, and after isolation with ether it crystallized from ethyl alcohol in prisms, m.p. 137–138° C.

O-Ethyl-*bis*-(4-nitrophenyl)-thiophosphate (IV) was heated at 150° C. for 5 hr. The dark coloured viscous residual liquid crystallized from ethyl alcohol in rectangular prisms, m.p. 104–106° C. which was raised to m.p. 109–110° C. by further crystallizations. This compound did not depress the m.p. of the product obtained by heating parathion. Treatment with fuming nitric acid at 0° C. gave a solid which crystallized from ethyl alcohol in prisms, m.p. 134–136° C. which was undepressed by admixture with the nitro-derivative obtained above.

Pure parathion m.p. 6° C. (Albright and Wilson, R. 7543. Found: thiono-sulphur, in terms of diethyl-(4-nitrophenyl)-thiophosphate, 112·7, 110·5 %; after ignition and washing with 3·6 % HCl 102·2, 101·4 %) was heated at 150° C. The amounts of unchanged compound after 10 and 15 hr. were 55 and 19·6 % respectively, after 18 hr. conversion was complete and after a further 15 hr. an extremely viscous black material remained.

O:S-Diethyl-(4-nitrophenyl)-thiophosphate ((II); Albright and Wilson, R. 7557), when heated for 15 hr. at 150° C. became dark and viscous, the product being insoluble in acetone.

O:O-Dimethyl-(4-nitrophenyl)-thiophosphate (Albright and Wilson, R. 7679) gave the following thiono-sulphur values for heat-treatment periods of 0, 1, 2½,

3 and 6 hr.: 114·4, 94·3, 80·3, 69·0 and 0 %. The original high value for dimethyl-parathion was found to be due to the coprecipitation of $Ba(NO_3)_2$ with $BaSO_4$, a $\equiv P=S$ value of 98·3 % being obtained after ignition and washing with HCl.

Toxicity of heat-treated samples

The results of toxicity tests, using *Calandra granaria*, for the heat-treated samples are given in Table 2. Test 49·5 shows the loss of potency of parathion with prolonged heat treatment, the resulting mixture being non-toxic after 24 hr. The crystalline compound isolated after heating parathion (presumably S-ethyl-*bis*-(4-nitrophenyl)-thiophosphate) was tested and as expected proved non-toxic. The thiono-sulphur values (calculated as diethyl-(4-nitrophenyl)-thiophosphate) for 0, 1, 7 and 24 hr. were 81·0, 75·8, 43·7, 0·0 %, respectively, and these are seen to be closely related to the corresponding relative potencies of 1·00:0·92:0·43:0·00. The computation of a single regression line ($\chi^2_9=13{\cdot}07$) on the basis of dose $\equiv$ weight deposit $\times$ thiono-sulphur content substantiates the conclusion that insecticidal activity was highly correlated with the thiono-sulphur content of the samples.

Both the ethyl-*bis*-(4-nitrophenyl)-thiophosphate and the compound which was obtained from it by heat treatment (S-ethyl-*bis*-(4-nitrophenyl)-thiophosphate) were non-toxic.

Pure O:O-diethyl-(4-nitrophenyl)-thiophosphate also lost potency after heating and a deposit of 3 mg. per filter-paper of the treated sample (thiono-sulphur value, 0·0) gave 50 % kill against the unheated sample deposit of 0·05 mg. The heated pure parathion was 0·62 times as toxic as its O:S diethyl isomer; the isomer after heat treatment of 15 hr. was apparently decomposed and the resulting products showed no insecticidal activity.

The effects of heat treatment on toxicity with O:O-dimethyl-(4-nitrophenyl)-thiophosphate were similar to those with parathion. The relative potencies of 1·00: 0·91:0·59:0·03 for heat treatments of 0, 1, 3 and 6 hr. duration, when compared with the corresponding thiono-sulphur values of 114·4, 95·1, 54·1 and 4·4 again indicate a pronounced relationship between toxicity and $\equiv P=S$ content.

Toxicity of isomers

The O:S-diethyl and O:O-diethyl isomers of parathion (II, III) were tested against parathion and also against para-oxon ((VI), diethyl-(4-nitrophenyl)-phosphate). These tests were carried out at different times and with different batches of *Calandra*. Inspection of Table 2 (tests 49·21–49·26) shows that the slope and position of the regression lines fluctuated appreciably. The data, however, could be fitted by a series of parallel lines (Table 4). The four compounds had been tested each against the other so that an analysis of variance (Table 5) could be made on the log (ED 50)s using a balanced incomplete block design (Yates, 1936). The relative potencies obtained from the corrected means ($\bar{m}$) are in good agreement with those obtained from individual lines (Table 2).

TABLE 2. *Organophosphorus compounds. Dosage-mortality data*

Test	Compound	Equation	b	$m \pm$	M	Relative potency	5 % Fid. limit
49·21	Parathion	$Y = 8{\cdot}97x + 2{\cdot}269$	$8{\cdot}97 \pm 0{\cdot}534$	$0{\cdot}3041$	$-0{\cdot}922 \pm 0{\cdot}0141$	1·00	$0{\cdot}112 - 0{\cdot}128$
	Para-oxon	$Y = 8{\cdot}97x - 6{\cdot}000$		$1{\cdot}2261$		1·120	
49·22	Parathion	$Y = 10{\cdot}65x + 4{\cdot}274$	$10{\cdot}65 \pm 0{\cdot}675$	$0{\cdot}069$	$-1{\cdot}438 \pm 0{\cdot}0129$	1·000	$0{\cdot}034 - 0{\cdot}039$
	O:O-diethyl-S-(4-nitrophenyl)-thiophosphate	$Y = 10{\cdot}65x - 11{\cdot}048$		$1{\cdot}507$		0·036	
49·23	Parathion	$Y = 9{\cdot}33x + 3{\cdot}573$	$9{\cdot}33 \pm 0{\cdot}607$	$0{\cdot}153$	$-1{\cdot}269 \pm 0{\cdot}0158$	1·000	$0{\cdot}050 - 0{\cdot}058$
	O:S-diethyl-O-(4-nitrophenyl)-thiophosphate	$Y = 9{\cdot}33x - 8{\cdot}262$		$1{\cdot}422$		0·054	
49·24	Para-oxon	$Y = 10{\cdot}15x - 0{\cdot}985$	$10{\cdot}15 \pm 0{\cdot}725$	$0{\cdot}589$	$-0{\cdot}413 \pm 0{\cdot}0148$	1·000	$0{\cdot}361 - 0{\cdot}413$
	O:O-diethyl-S-(4-nitrophenyl)-thiophosphate	$Y = 10{\cdot}15x - 5{\cdot}178$		$1{\cdot}002$		0·386	
49·25	Para-oxon	$Y = 9{\cdot}85x - 3{\cdot}215$	$9{\cdot}85 \pm 0{\cdot}642$	$0{\cdot}834$	$-0{\cdot}316 \pm 0{\cdot}0147$	1·000	$0{\cdot}452 - 0{\cdot}516$
	O:S-diethyl-O-(4-nitrophenyl)-thiophosphate	$Y = 9{\cdot}85x - 6{\cdot}331$		$1{\cdot}150$		0·483	
49·26	O:O-diethyl-S-(4-nitrophenyl)-thiophosphate	$Y = 12{\cdot}24x - 13{\cdot}861$	$12{\cdot}24 \pm 0{\cdot}815$	$1{\cdot}541$	$-0{\cdot}176 \pm 0{\cdot}0130$	0·667	$0{\cdot}628 - 0{\cdot}706$
	O:S-diethyl-O-(4-nitrophenyl)-thiophosphate	$Y = 12{\cdot}24x - 11{\cdot}705$		$1{\cdot}365$		1·000	
50·6	O:O-diethyl-O-(4-nitrophenyl)-thiophosphate	$Y = 10{\cdot}43x - 1{\cdot}495$	$10{\cdot}43 \pm 0{\cdot}646$	$0{\cdot}623$	$-0{\cdot}075 \pm 0{\cdot}0138$	1·000	$0{\cdot}791 - 0{\cdot}895$
	Parathion	$Y = 10{\cdot}43x - 2{\cdot}277$		$0{\cdot}698$		0·841	
50·1	Parathion	$Y = 10{\cdot}97x - 13{\cdot}999$	$10{\cdot}97 \pm 1{\cdot}025$	$1{\cdot}732 \pm 0{\cdot}0037$			
	O:O-dimethyl-O-(4-nitrophenyl)-thiophosphate	$Y = 7{\cdot}56x - 0{\cdot}165$	$7{\cdot}56 \pm 0{\cdot}631$	$0{\cdot}683 \pm 0{\cdot}0119$	—	—	—
50·2	O:O-di-(2-chloroethyl)-O-(4-nitrophenyl)-thiophosphate	$Y = 2{\cdot}94x - 2{\cdot}226$	$2{\cdot}94 \pm 0{\cdot}216$	$2{\cdot}456 \pm 0{\cdot}0259$			
	Parathion	$Y = 6{\cdot}53x + 1{\cdot}562$	$6{\cdot}53 \pm 0{\cdot}529$	$0{\cdot}526 \pm 0{\cdot}0129$	—	—	—
49·5	Parathion. Heat treatment for 24 hr.	—	—	—	—	—	—
	Parathion. Heat treatment for 7 hr.	$Y = 5{\cdot}03x - 2{\cdot}362$	—	$1{\cdot}465$	$-0{\cdot}369 \pm 0{\cdot}0451$	$0{\cdot}428$	$0{\cdot}349 - 0{\cdot}524$
	Parathion. Heat treatment for 1 hr.	$Y = 5{\cdot}03x - 0{\cdot}688$	$5{\cdot}03 \pm 0{\cdot}507$	$1{\cdot}132$	$-0{\cdot}036 \pm 0{\cdot}044$	$0{\cdot}921$	$0{\cdot}754 - 1{\cdot}100$
	Parathion. Heat treatment for 0 hr.	$Y = 5{\cdot}03x - 0{\cdot}508$	—	$1{\cdot}096$	—	1·000	—
50·28	O:O-diethyl-O-(4-nitrophenyl)-thiophosphate. Heated for 18 hr.	$Y = 13{\cdot}66x - 2{\cdot}081$	$13{\cdot}66 \pm 1{\cdot}044$	$0{\cdot}517$	$-0{\cdot}211 \pm 0{\cdot}0124$	0·615	$0{\cdot}582 - 0{\cdot}650$
	O:S-diethyl-O-(4-nitrophenyl)-thiophosphate. Heated for 0 hr.	$Y = 13{\cdot}66x + 0{\cdot}824$		$0{\cdot}306$		1·000	
50·5	O:O-diethyl-O-(4-nitrophenyl)-thiophosphate. Heated for 6 hr.	$Y = 6{\cdot}63x - 11{\cdot}540$	—	$2{\cdot}495$	$-1{\cdot}601 \pm 0{\cdot}0191$	$0{\cdot}025$	$0{\cdot}023 - 0{\cdot}027$
	O:O-diethyl-O-(4-nitrophenyl)-thiophosphate. Heated for 3 hr.	$Y = 6{\cdot}63x - 2{\cdot}457$	$6{\cdot}63 \pm 0{\cdot}030$	$1{\cdot}125$	$-0{\cdot}231 \pm 0{\cdot}0187$	$0{\cdot}588$	$0{\cdot}540 - 0{\cdot}640$
	O:O-diethyl-O-(4-nitrophenyl)-thiophosphate. Heated for 1 hr.	$Y = 6{\cdot}63x - 1{\cdot}205$		$0{\cdot}936$	$-0{\cdot}042 \pm 0{\cdot}0187$	$0{\cdot}908$	$0{\cdot}834 - 0{\cdot}986$
	O:O-diethyl-O-(4-nitrophenyl)-thiophosphate. Heated for 0 hr.	$Y = 6{\cdot}63x - 0{\cdot}927$	—	$0{\cdot}894$	—	1·000	—

TABLE 3. *Analysis of χ^2 for data of Table 2*

Test	Parallelism of regressions D.F.	Parallelism of regressions Sum of squares	Residual heterogenity D.F.	Residual heterogenity Sum of squares	Total D.F.	Total Sum of squares
49·21	1	0·044	8	12·230	9	12·274
49·22	1	0·779	7	5·160	8	5·939
49·23	1	3·652	7	2·258	8	5·910
49·24	1	0·046	6	5·602	7	5·648
49·25	1	1·319	5	2·611	6	3·930
49·26	1	0·019	4	1·078	5	1·097
50·6	1	0·054	5	2·710	6	2·764
50·1	Not parallel	—	—	—	—	—
50·2	Not parallel	—	—	—	—	—
49·5	2	0·429	3	6·967	5	7·396
50·28	1	0·265	3	2·140	4	2·405
50·5	3	5·834	15	13·704	18	19·538

TABLE 4. *Combined dosage-mortality data for tests* 49·21–49·26

Test	Equation	m
49·21	$Y = 10·02x +$	1·939 0·3055
		−7·286 1·2261
49·22		4·325 0·0674
		−10·095 1·5064
49·23		3·464 0·1533
		−9·257 1·4228
49·24		−0·905 0·5893
		−5·042 1·0021
49·25		−3·355 0·8337
		−6·523 1·1500
49·26		−10·446 1·5415
		−8·692 1·3664

Analysis of χ^2	D.F.	Sum of squares
Parallelism of regressions	11	10·609
Residual heterogenity	37	27·685
Total	48	38·294

O:O-Dimethyl-(4-nitrophenyl)-thiophosphate was tested against parathion and was found to have a considerably greater activity. This relatively higher potency was obtained consistently and it was suspected that the dimethyl compound was exerting a strong fumigant action, which it was known to possess, that could not be eliminated in the type of test used. The slopes of the regression lines were significantly different and the relative toxicities at 5, 50 and 95 % levels of kill being 13·09, 11·20 and 9·59.

O:O-Di-(2-chloroethyl)-O-(4-nitrophenyl)-thiophosphate (Pal Chemicals Ltd., no. P. 11) was considerably less toxic than parathion and apparently much slower in action. The relative potencies at 5, 50 and 95 % levels of kill were 0·024, 0·012 and 0·006.

Estimates of relative potency of the various compounds were made using a 'purified' parathion as standard, and this was assayed against a pure crystalline sample of diethyl-(4-nitrophenyl)-thiophosphate, when this became available; the potency being 0·85 (5 % fiducial limits of 0·79–0·90).

TABLE 5. *Analysis of variance: Tests* 49·21–49·26

Insecticides \ Tests	21	22	23	24	25	26	Totals	Q	V	Corrected means
Parathion	0·3055	0·0674	0·1533	—	—	—	0·5262	−3·6291	−0·9073	0·0231
Para-oxon	1·2261	—	—	0·5893	0·8337	—	2·6491	0·1915	0·0479	0·9783
O:O-diethyl-S-(4-nitrophenyl)-thiophosphate	—	1·5064	—	1·0021	—	1·5415	4·0500	2·0269	0·5067	1·4371
O:S-diethyl-O-(4-nitrophenyl)-thiophosphate	—	—	1·4228	—	1·1500	1·3664	3·9392	1·4107	0·3527	1·2831
Totals	1·5316	1·5738	1·5761	1·5914	1·9837	2·9079	11·1645	0	0	—

Item	D.F.	Sum of squares	Mean square
Insecticides	3	2·41318	0·8044
Tests	5	0·72795	0·1456
Error	3	0·00235	0·00078
Total	11	3·14348	—

	m	*M*	Rel. potency
Parathion	0·0231	—	1·000
Para-oxon	0·9783	−0·9552	0·111
O:O-diethyl-S-(4-nitrophenyl)-thiophosphate	1·4371	−1·4140	0·039
O:S-diethyl-O-(4-nitrophenyl)-thiophosphate	1·2831	−1·2600	0·055

Standard error of the difference between treatment means is 0·0279.

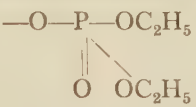
Discussion

The isomerization of O:O-dimethyl- and O:O-diethyl-O-(4-nitrophenyl)-thiophosphate by heating at temperatures of 140° and above has been confirmed and from the chemical and biological evidence obtained it was concluded that parathion (I) is rearranged to form (II). Further heating of this isomer resulted in thermal decomposition with subsequent loss of toxicity. The non-toxic crystalline compound, m.p. 111–111·5° C., obtained when a commercial preparation of parathion was heated, was not identical with either of the parathion isomers (II), (III). A consideration of analytical and molecular weight data indicated a more complex structure, and infra-red photography favoured formula (V). This was also supported by the isolation of an identical substance from heat-treated O-ethyl-*bis*-(4-nitrophenyl)-thiophosphate (VI). Since this product (IV) was not obtained when pure parathion was heated, it was concluded that its formation in the earlier experiments was due to the presence of the precursor (V) in the impure sample, and that no disproportionation of parathion had taken place.

Para-oxon, O:S-diethyl- and O:O-diethyl-parathion isomers although highly toxic to *C. granaria* have low relative potencies in comparison to parathion; the parallelism of their regression lines indicates similarity in the mode of action. Dimethyl-(4-nitrophenyl)-thiophosphate was considerably more toxic than parathion and although the slopes of their regression lines were significantly different it was thought that their biological activity was probably similar since the visual effects were more or less identical. It was suspected that this relatively high value for the potency and the difference of slope was due to fumigant action by the dimethyl compound. The low slope and toxicity together with an apparent different action judged visually indicates that the dichloroethyl-(4-nitrophenyl)-thiophosphate has a different mode of action.

Little has been published concerning the relationship between chemical structure and the insecticidal activity of parathion analogues. Metcalf & Marsh (1949) have investigated changes both in the phenyl nucleus and in the aliphatic portion of the molecule, but there appears to be no information regarding structural isomers.

Martin (1947) has suggested that an important toxophoric grouping is



Schrader (1947) found that the effect of substituting P=S for the P=O group resulted in a decrease of mammalian toxicity but no great change of insecticidal activity. In the present work sulphur and oxygen do not appear to be mutually replaceable, and the optimum arrangement of atoms around the central phosphorus atom is obtained in (I); the isomers involving the substitution of P=O for P=S are less toxic.

Modification of the size of the parathion molecule, e.g. replacement of an ethyl group by (4-nitrophenyl) as in (IV) and (V) or both ethyl groups by 2-chloroethyl as in (VII) results in loss of toxicity. Whether this is because the resultant structures possess less free energy than the original parathion (Brauer, 1948) is a matter for conjecture, though in all three cases stability has also increased. The thermal conversion of the less stable isomer (I) to the more stable compound (II) with subsequent loss of activity would also appear to substantiate this premise.

Metcalf & Marsh (1949) have reported that compound (VIII) has the same order of toxicity as parathion, when tested against worker bees and house-flies. This appears to be an exception, no mention being made of its stability.

The authors are indebted to Dr H. Martin for his interest and advice in carrying out this work, to Dr L. J. Bellamy for taking infra-red photographs, and to Mr R. F. Batt for the sulphur analyses.

REFERENCES

Bennett, S. H., Martin, H., Stringer A. & Woodcock, D. (1948). The qualitative examination of insecticidal properties. Progress Report, 1948. *Rep. agric. hort. Res. Sta., Bristol*, p. 138.

Brauer, R. W. (1948). Inhibition of the cholinesterase activity of human blood plasma and erythrocyte stromata by alkylated phosphorus compounds. *J. Pharmacol.* **92**, 162.

Emmett, W. J. & Jones, H. O. (1911). Isomeric monothiophosphates. *J. chem. Soc.* **99**, 713.

Finney, D. J. (1947). *Probit Analysis*. Cambridge University Press.

Martin, H. (1947). Relationship between chemical constitution and insecticidal properties among chlorinated hydrocarbons and organic phosphates. *XI Int. Congress Pure and Appl. Chem.* London, 17–24 July 1947.

Metcalf, R. L. & Marsh, R. B. (1949). Studies of the mode of action of parathion and its derivatives and their toxicity to insects. *J. econ. Ent.* **42**, 721.

Schrader, G. (1947). B.I.O.S. Final Report no. 1808.

Stringer, A. (1949). A simple method for assaying contact toxicities of insecticides, with results of tests of some organic compounds against *Calandra granaria* L. *Ann. appl. Biol.* **36**, 213.

Yates, F. (1936). Incomplete randomized blocks. *Ann. Eugen., Camb.*, **7**, 121.

(*Received* 5 *July* 1950)

THE CONTROL OF WIREWORM BY GAMMA BENZENE HEXACHLORIDE: THE DEVELOPMENT OF A SEED DRESSING FOR CEREALS

By H. R. JAMESON, B.Sc., A.R.I.C., A.M.S.E., F. J. D. THOMAS, A.R.C.S., B.Sc. and C. C. TANNER, B.Sc., Ph.D.

Hawthorndale Laboratories, Jealott's Hill Research Station, Bracknell, Berks

(With 8 Text-figures)

Gamma benzene hexachloride has been used successfully as a seed dressing in field trials for wireworm control. For application to cereals at 2 oz./bushel, the optimum concentration of γ-isomer was 20–30 % in a dressing which advantageously included an organomercurial seed disinfectant.

Risks to germination and plant growth were thoroughly explored in the field. The 20 and 30 % dressings were safe; over-strength dressings containing 50 or 70 % γ-B.H.C. did not reduce grain yields significantly.

Seed dressed with 20 or 30 % γ-B.H.C. germinated normally after storage for 12 months. When seed was dressed at 4 oz./bushel with 35 % γ-B.H.C. and 1 % mercury as organomercurial, germination was delayed but total emergence was not affected.

Introduction

Earlier work (Jameson, Thomas & Woodward, 1947) had shown that promising protection against wireworm attack could be obtained by dressing seed oats with 8 % γ-B.H.C. at the impractical rate of 0·8 % on the weight of the seed (5 oz./bushel). Subsequent work has led to the development of a cereal seed dressing, for application at the normal rate of 2 oz./bushel to oats sown at 4–6 bushels to wheat and sown at 3 bushels/acre. The dressing advantageously includes an organomercurial.

The present paper is limited to the following important aspects of the work:

(*a*) Effect of varying the γ-B.H.C. content of the dressing, with organomercurial constant at 1 % mercury, on plant stands and grain yields, under conditions of heavy wireworm infestation.

(*b*) Effect of such dressings on seed germination and plant growth.

(*c*) Effect of storage on the germination and growth of dressed seeds.

Experimental

High grade γ-B.H.C., approx. 98 %, was used in the preparation of the dressings which also contained organomercurial in the same form and amount as in Agrosan GN. Formulations of these dual-purpose or 'gamma B.H.C./mercurial' dressings*

* B.P. 629670, I.C.I. Ltd. and F. L. Sharp, covers pulverulent γ-B.H.C./organomercurial seed dressings such as Mergamma A, B, C, etc.

were selected for field trial after tests, not detailed in this paper, had shown that they adhered well to the seed and did not impair germination or the growth of seedlings.

Sites were selected on the basis of information relating to wireworm populations, chiefly provided by Local Advisory Entomologists (later the N.A.A.S.). The fields were mostly first year after grass and the populations of wireworms, over 5 mm. in length, ranged from 500,000/acre to more than 1,500,000/acre. The experiments, which generally comprised randomized strips of 2-drill width, usually crossing the field, were laid down by I.C.I. staff.

Plant populations were determined either by visual appraisal or by taking counts of seedlings, before tillering, in twenty to thirty random 1-yard lengths of drill row in each strip.

Yields of grain and straw were based on the produce of ten areas, each 1 yard square, spaced at regular intervals along each strip. In a few trials whole strips were combine harvested.

The data were analysed statistically; coefficients of variation and significant differences at the 5 and 1 % levels are recorded. Significant regressions of plant stands, grain and straw yields on the percentage of gamma B.H.C. in the dressings are also noted.

1947 *Trials*

With the organomercurial content of the dressing constant at 1 % mercury, the effect of varying the γ-B.H.C. content from 0 to 35 % was examined in six field trials laid down in the neighbourhood of Hawthorndale in the spring of 1947.

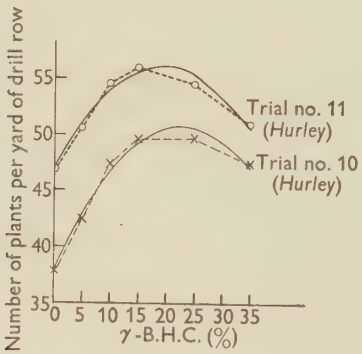


Fig. 1.

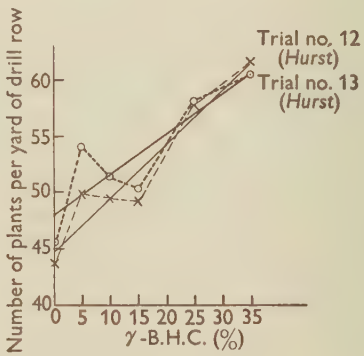


Fig. 2.

Plant populations just before tillering are given in Table 1; the effect of increasing γ-B.H.C. content is well shown in Figs. 1 and 2. In trials 10 and 11* maximum plant populations were secured with seed dressings containing 20–25 % γ-B.H.C.

* Earlier trials of similar type, 1–9, served to show that the optimum γ-B.H.C. level under moderate to heavy wireworm attack was within the range of 10–35 % for application at 2 oz./bushel, and that organomercurial could be safely included.

TABLE 1. *Effect of varying the γ-B.H.C. content of seed dressings in the control of wireworm. Spring sown oats*, 1947

(*a*) Actual data

			Seed dressings at 2 oz./bushel containing 1 % Hg.						Statistical criteria	
			0 % γ-B.H.C. (control	5 % γ-B.H.C.	10 % γ-B.H.C.	15 % γ-B.H.C.	25 % γ-B.H.C.	35 % γ-B.H.C.	Coeff. of var. (%)	Regression
Light wireworm attack	Trial no. 10	Plants/1 yd. row 43 days after sowing	37·8	42·4	47·4	49·6	49·6	47·3	8·3	2nd degree curve, max. at 20 % γ-B.H.C.
		Yield in cwt./acre Straw	24·0	25·9	24·7	25·9	25·7	26·6	5·6	Linear regression
		Yield in cwt./acre Grain	19·6	21·1	20·5	21·8	21·2	22·0	6·8	Linear regression
	Trial no. 11	Plants/1 yd. row 34 days after sowing	46·8	50·6	54·6	56·0	54·5	50·8	5·6	2nd degree curve, max. at 23 % γ-B.H.C.
		Yield in cwt./acre Straw	20·1	19·1	21·6	21·0	20·3	22·8	7·0	Linear regression
		Yield in cwt./acre Grain	14·9	14·3	16·4	15·5	15·4	16·7	8·7	None
Heavy wireworm attack	Trial no. 12	Plants/1 yd. row 25 days after sowing	43·7	49·8	49·4	49·1	57·7	61·5	3·9	Linear regression
		Growth mark 28 days after sowing	7·3	8·3	7·9	8·1	9·9	10·0	—	—
		Yield in cwt./acre Straw	29·1	33·4	34·0	35·4	33·7	36·6	7·0	Linear regression
		Yield in cwt./acre Grain	14·5	17·7	18·8	19·3	20·8	21·6	8·1	2nd degree curve, max. at 32 % γ-B.H.C.
	Trial no. 13	Plants/1 yd. row 25 days after sowing	45·6	54·1	51·3	50·2	58·1	60·4	5·1	Linear regression
		Growth mark 28 days after sowing	7·3	8·4	8·4	8·4	9·1	9·4	—	—
		Yield in cwt./acre Straw	37·6	38·4	37·6	38·3	42·5	42·8	9·8	Linear regression
		Yield in cwt./acre Grain	17·2	19·1	18·2	20·1	23·2	22·9	9·6	Linear regression

(*b*) Data calculated on basis of control = 100

			Seed dressings at 2 oz./bushel containing 1 % Hg						Statistical criteria	
			0 % γ-B.H.C. (control)	5 % γ-B.H.C.	10 % γ-B.H.C.	15 % γ-B.H.C.	25 % γ-B.H.C.	35 % γ-B.H.C.	Coeff. of var. (%)	Regression
Light wireworm attack	Trial no. 10	Plants/unit length of row 43 days after sowing	120	112	125	131	131	125	8·3	2nd degree curve, max. at 20 % γ-B.H.C.
		Yield Straw	100	108	103	108	107	111	5·6	Linear regression
		Yield Grain	100	108	105	111	108	112	6·8	Linear regression
	Trial no 11	Plants/unit length of row 34 days after sowing	100	108	117	120	116	108	5·6	2nd degree curve, max. at 23 % γ-B.H.C.
		Yield Straw	100	95	108	105	101	114	7·0	Linear regression
		Yield Grain	100	96	110	104	103	112	8·7	None
Heavy wireworm attack	Trial no. 12	Plants/unit length of row 25 days after sowing	100	114	113	112	132	141	3·9	Linear regression
		Growth mark 28 days after sowing	100	114	108	111	136	137	—	—
		Yield Straw	100	115	117	122	116	126	7·0	Linear regression
		Yield Grain	100	122	129	133	143	149	8·1	2nd degree curve, max. at 32 % γ-B.H.C.
	Trial no. 13	Plants/unit length of row 25 days after sowing	100	119	113	110	127	133	5·1	Linear regression
		Growth mark 28 days after sowing	100	115	115	115	125	129	—	—
		Yield Straw	100	102	100	102	113	114	9·8	Linear regression
		Yield Grain	100	111	106	117	135	134	9·6	Linear regression

In trials 12 and 13, plant populations increased steadily throughout the range of B.H.C.-content covered. Wireworm attack after plant counts were taken was light in trials 10 and 11, but serious in trials 12 and 13.

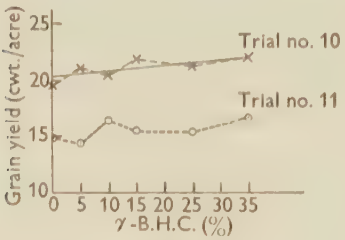


Fig. 3.

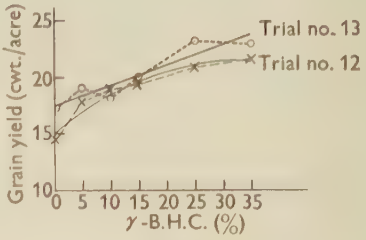


Fig. 4.

Grain and straw yield estimates are given in Table 1 and plotted in Figs. 3 and 4. In trials 10, 12 and 13, but not in 11, the regressions of grain yield on the percentage of γ-B.H.C. were positive and significant at the 5 % level. The regressions were linear, except in trial 12, for which a flat maximum was reached at 32 % γ-B.H.C., nearly at the end of the range. The regressions of straw yield on the percentage of γ-B.H.C. were significant and linear for all trials.

1948 *Trials*

In order to confirm previous findings seven additional trials were laid down in autumn 1947 and spring 1948 with dressings containing γ-B.H.C. in the range 0 30 %, and 1 % mercury as organomercurial. Wireworm populations ranged from 440,000 to 800,000/acre but heavy attack did not develop.

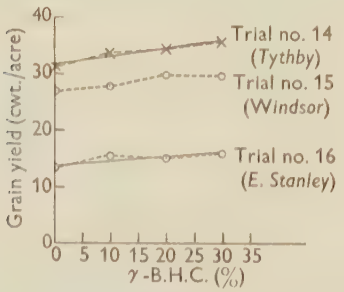


Fig. 5.

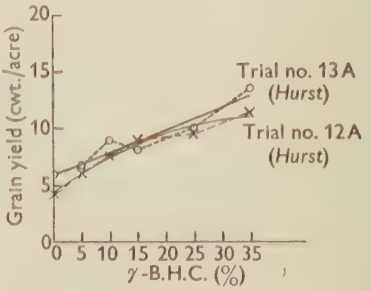


Fig. 6.

Grain and straw yields for trials 14, 15 and 16 are given in Table 2 and plotted in Fig. 5. Plant populations were taken for one trial only, no. 15. For all data, significant positive regressions were found on γ-B.H.C. content, except for trial no. 15 where wireworm attack was very light and grain yields failed to reach

TABLE 2. *Effect of varying the γ-B.H.C. content of γ-B.H.C./mercurial seed dressing in the control of wireworm. Autumn-sown (1947) and spring-sown (1948) cereals*

			(*a*) Actual data. Seed dressings at 2 oz./bushel containing 1 % Hg.				Statistical criteria	
			0 % γ-B.H.C. (control)	10 % γ-B.H.C.	20 % γ-B.H.C.	30 % γ-B.H.C.	Coeff. of var. (%)	Regression
Trial no. 14	Wheat sown, autumn 1947	Grain yield in cwt./acre	31·3	33·8	34·4	35·5	5·6	Linear regression
Trial no. 15	Wheat sown, spring 1948	Plants/1 yd. row 6 wk. after sowing	39·9	55·0	66·8	65·5	10·8	2nd degree curve, max. at 26 % γ-B.H.C.
		Growth mark 8 wk. after sowing	5·3	9·0	9·8	9·8	—	—
		Yield in cwt./acre: Straw	50·3	55·2	62·8	59·8	4·3	2nd degree curve, max. at 23 % γ-B.H.C.
		Yield in cwt./acre: Grain	27·0	27·9	29·7	29·6	7·2	None
Trial no. 16	Oats sown, spring 1948	Grain yield in cwt./acre	13·3	15·4	15·1	15·8	8·0	Linear regression
		(*b*) Data calculated on basis of control = 100						
Trial no. 14	Wheat sown, autumn 1947	Grain yield	100	108	110	113	5·6	Linear regression
Trial no. 15	Wheat sown, spring 1948	Plants/unit length of row 6 wk. after sowing	100	138	168	165	10·8	2nd degree curve, max. at 23 % γ-B.H.C.
		Growth mark 8 wk. after sowing	100	170	185	185	—	—
		Yield: Straw	100	110	125	119	4·3	2nd degree curve, max. at 23 % γ-B.H.C.
		Yield: Grain	100	103	110	110	7·2	None
Trial no. 16	Oats sown, spring 1948	Grain yield	100	116	114	119	8·0	Linear regression

TABLE 3. *Effect in* 1948 *of sowing dressed seed in spring*, 1947

			(*a*) Actual data: Seed dressings at.2 oz./bushel containing 1 % Hg						Statistical criteria	
			0 % γ-B.H.C. (control)	5 % γ-B.H.C.	10 % γ-B.H.C.	15 % γ-B.H.C.	25 % γ-B.H.C.	35 % γ-B.H.C.	Coeff. of var. %	Regression
Trial no. 12A (1948 sowing)	Yield of oats cwt./acre	Straw	8·8	11·4	18·8	23·6	22·9	26·0	23·8	2nd degree curve, max. at 30 % γ-B.H.C.
		Grain	4·2	6·0	7·6	9·0	9·5	11·3	18·1	2nd degree curve, max. at 39 % γ-B.H.C.
Trial no. 13A (1948 sowing)	Yield of oats cwt./acre	Straw	11·0	12·8	16·0	13·9	18·8	27·7	33·2	Linear regression
		Grain	5·9	6·7	8·9	8·1	10·1	13·6	32·1	Linear regression
			(*b*) Data calculated on basis of control = 100							
Trial no. 12A (1948 sowing)	Yield of oats	Straw	100	130	214	268	260	295	23·8	2nd degree curve, max. at 30 % γ-B.H.C.
		Grain	100	143	181	214	226	270	18·1	2nd degree curve, max. at 39 % γ-B.H.C.
Trial no. 13A (1948 sowing)	Yield of oats	Straw	100	116	146	127	171	251	33·2	Linear regression
		Grain	100	114	151	137	171	230	32·1	Linear regression

significance. The regressions were linear except those for plant populations and straw yields in no. 15, in which flat maxima were reached at about 25 % γ-B.H.C.

Effect of γ-B.H.C. seed dressings in later seasons

Evidence was found that the effect of a γ-B.H.C. dressing can last into the second season. Thus, trials 12 and 13, laid down in spring, 1947, were followed by spring oats in 1948 (trials 12A and 13A) sown without B.H.C. treatment. Yield data are given in Table 3 and plotted in Fig. 6. The regressions of grain and straw yields on the γ-B.H.C. content were significant and positive in both trials, with a flat maximum near the end of the range for 12A and a linear relationship for 13A.

Phytotoxicity trials 1947–8

The object of these trials was to test the safety to plants of preferred dressings containing 20-30 % γ-B.H.C. and 1 % mercury as organomercurial under diverse conditions of soil and climate. To examine possible phytotoxic effects the gamma B.H.C. range was extended to 70 %.

Ten trials on winter oats or wheat were laid down in Somerset, Sussex, Berkshire, Warwick and Durham on land that had been arable for some time and was as free as possible from wireworm. A site used in Cheshire was not so free. The layout consisted of 50-yard randomized, replicated strips. Since differential emergence rates were expected, plant counts were taken every 2 or 3 days where growth was rapid, and less frequently where it was slow. Total emergence was determined after some weeks.

Results are given in Tables 4 and 5 plotted in Figs. 7 and 8. Growing conditions strikingly affected phytotoxicity. The effect was greatest on wheat seedlings growing rapidly, no. 21. In this trial, even the 20 % γ-B.H.C. dressings slightly retarded emergence, but the effect, though initially significant, disappeared after a few days. The effect of the 30 % γ-B.H.C. dressing was similar and that of the 50 %, which was more pronounced, disappeared about 10 weeks after sowing. The 70 % γ-B.H.C. dressing very seriously retarded emergence and the plots were inferior to the controls 10 weeks after sowing. These visual assessments were confirmed by plant counts. In the other trials results were similar wherever growing conditions were good, but there was no phytotoxicity where growth was very slow, in no. 25 for example.

Grain yields were not significantly reduced even at the 70 % level of γ-B.H.C. On the contrary, in nos. 19 and 24, yields significantly increased slightly with increasing percentage of γ-B.H.C. Straw yields reflected grain yields, except in no. 20 where regression was negative. In no. 23 straw yield reached a maximum at about 30 % γ-B.H.C.

TABLE 4. *Effect of γ-B.H.C./mercurial seed dressings on cereal stands under conditions of little or no wireworm population, 1947–8*

(*a*) Actual data. (Plants per 1 yard drill row)

	Growing conditions just after sowing	Days after sowing	0 % γ-B.H.C. 1 % Hg	20 % γ-B.H.C. 1 % Hg	30 % γ-B.H.C. 1 % Hg	50 % γ-B.H.C. 1 % Hg	70 % γ-B.H.C. 1 % Hg	Statistical criteria: Coeff. of var. (%)	Statistical criteria: Regression
Trial no. 17, Jealott's Hill (oats)	Rapid	12	40·5	42·2	36·2	34·1	—	4·7	Linear regression
		15	47·7	46·5	49·8	48·3	—	7·9	None
Trial no. 18, Warwick (wheat)	Slow	21	8·9	8·8	10·5	7·1	—	19·0	2nd degree curve, max. at 20 % γ-B.H.C.
		24	7·4	8·5	9·2	6·4	—	6·8	2nd degree curve, max. at 22 % γ-B.H.C.
		37	8·6	8·1	9·1	6·7	—	6·5	None
		53	20·2	20·6	23·4	22·9	—	8·4	Linear regression
Trial no. 19, Jealott's Hill (wheat)	Slow to normal	16	16·6	17·9	18·4	13·9	10·2	14·2	2nd degree curve, max. at 19 % γ-B.H.C.
		19	17·2	19·8	19·3	17·4	13·8	16·1	2nd degree curve, max. at 26 % γ-B.H.C.
		26	23·4	24·8	27·2	24·9	19·2	7·9	2nd degree curve, max. at 29 % γ-B.H.C.
		33	30·5	29·5	29·3	29·5	24·7	7·4	Linear regression
Trial no. 20, Crewkerne (oats)	Very rapid	13	32·9	32·6	30·4	26·8	25·7	3·6	Linear regression
		16	37·4	34·8	35·5	32·4	31·8	5·2	Linear regression
		24	40·4	41·9	40·0	38·4	39·0	5·5	None
Trial no. 21, Crewkerne (wheat)	Very rapid	10	33·6	31·3	28·7	22·6	11·5	12·9	2nd degree curve, max. at 0 % γ-B.H.C.
		13	46·6	44·2	41·0	35·2	19·9	8·1	2nd degree curve, max. at 3 % γ-B.H.C.
		22	59·2	55·3	56·5	52·0	40·9	5·6	2nd degree curve, max. at 5 % γ-B.H.C.
Trial no. 22, Fernhurst (oats)	Very rapid	16	27·4	25·4	23·6	19·9	18·0	14·0	Linear regression
		19	34·8	35·6	30·8	25·4	25·4	5·5	Linear regression
		27	46·0	46·1	44·5	43·0	42·6	5·1	Linear regression
Trial no. 23, Fernhurst (wheat)	Very rapid	14	40·0	32·4	31·4	18·2	8·2	18·3	Linear regression
		17	47·2	43·0	39·9	26·8	10·7	9·3	2nd degree curve, max. at 0 % γ-B.H.C.
		25	54·0	52·0	49·6	42·8	26·9	4·3	2nd degree curve, max. at 6 % γ-B.H.C.
		38	52·2	53·5	51·6	46·2	33·6	6·7	2nd degree curve, max. at 15 % γ-B.H.C.
Trial no. 24, Haverton Hill (wheat)	Fairly slow	20	14·6	15·1	13·4	12·9	13·3	18·3	None
		27	19·0	20·5	18·5	18·4	18·8	12·2	None
		41	30·3	29·0	29·0	27·0	27·0	4·9	Linear regression
Trial no. 25, Haverton Hill (wheat)	Very slow	18	9·1	12·2	9·4	6·4	8·2	26·4	None
		22	14·8	15·3	13·5	13·6	13·7	26·9	None
		27	14·8	16·6	17·5	14·6	17·0	28·5	None
		82	53·2	58·3	58·4	60·0	61·8	7·0	Linear regression
Trial no. 26, Middlewich (oats)	Normal	25	44·9	37·5	35·6	36·2	34·2	10·6	Linear regression
		28	48·0	45·8	45·2	40·8	39·2	11·2	Linear regression
		41	56·1	57·6	57·3	54·8	53·7	5·5	None

(*b*) Data calculated on basis of control = 100

	Days after sowing	0 % γ-B.H.C. 1 % Hg	20 % γ-B.H.C. 1 % Hg	30 % γ-B.H.C. 1 % Hg	50 % γ-B.H.C. 1 % Hg	70 % γ-B.H.C 1 % Hg
Trial no. 17, Jealott's Hill (oats)	12	100	105	89	84	—
	15	100	98	104	101	—
Trial no. 18, Warwick (wheat)	21	100	100	118	80	—
	24	100	115	124	87	—
	37	100	94	106	78	—
	53	100	102	116	113	—
Trial no. 19, Jealott's Hill (wheat)	16	100	108	111	84	62
	19	100	115	112	101	80
	26	100	106	116	106	82
	33	100	97	96	97	81
Trial no. 20, Crewkerne (oats)	13	100	99	97	82	78
	16	100	93	95	87	85
	24	100	104	99	95	97
Trial no. 21, Crewkerne (wheat)	10	100	93	85	67	34
	13	100	95	88	76	43
	22	100	94	96	88	69
Trial no. 22, Fernhurst (oats)	16	100	93	86	73	66
	19	100	102	88	73	73
	27	100	100	97	94	93
Trial no. 23, Fernhurst (wheat)	14	100	81	78	45	21
	17	100	91	85	57	23
	25	100	96	92	79	50
	38	100	103	99	88	64
Trial no. 24, Haverton Hill (wheat)	20	100	103	92	88	91
	27	100	108	97	97	99
	41	100	97	97	90	90
Trial no. 25, Haverton Hill (wheat)	18	100	134	103	77	90
	22	100	103	91	92	93
	27	100	112	118	99	115
	82	100	110	110	113	115
Trial no. 26, Middlewich (oats)	25	100	84	79	81	76
	28	100	96	94	85	82
	41	100	103	102	98	96

TABLE 5. *Effect of γ-B.H.C./mercurial seed dressings on yield of cereals under conditions of little or no wireworm populations, 1947–8*

				Actual data: Seed dressings containing 1 % Hg and γ-B.H.C.						Statistical criteria	Data on basis control = 100				
				Nil γ-B.H.C.	20 % γ-B.H.C.	30 % γ-B.H.C.	50 % γ-B.H.C.	70 % γ-B.H.C.	Coeff. of var. (%)	Regression	Nil γ-B.H.C.	20 % γ-B.H.C.	30 % γ-B.H.C.	50 % γ-B.H.C.	70 % γ-B.H.C
Trial no. 17* Jealott's Hill	Oats	Yield	Grain	25·7	26·4	28·4	27·2	—	6·0	None	100	103	111	106	—
Trial no. 19, Jealott's Hill	Wheat	Yield cwt./acre	Straw	72·1	73·0	70·4	74·4	73·6	4·0	None	100	101	98	103	102
		Grain		37·4	36·8	37·1	39·5	38·7	3·9	Linear regression	100	98	99	106	104
Trial no. 20, Crewkerne	Oats	Yield	Straw	36·7	34·3	28·2	33·3	29·8	8·7	Linear regression neg.	100	93	77	91	81
		cwt./acre	Grain	28·0	27·2	26·0	27·9	26·8	8·5	None	100	97	93	100	96
Trial no. 21, Crewkerne	Wheat	Yield	Straw	39·6	43·5	36·5	41·8	34·6	16·1	None	100	110	92	106	87
		cwt./acre	Grain	22·2	23·0	21·6	24·1	21·1	13·0	None	100	104	97	109	95
Trial no. 22, Fernhurst	Oats	Yield	Straw	30·4	31·8	30·1	28·8	28·2	15·4	None	100	105	99	95	93
		cwt./acre	Grain	17·8	19·8	19·2	18·0	17·2	10·4	None	100	111	108	101	97
Trial no. 23, Fernhurst	Wheat	Yield cwt./acre	Straw	32·6	36·1	36·9	34·5	29·5	9·0	2nd degree curve, max. at 30 % γ-B.H.C.	100	111	113	106	91
			Grain	12·7	13·5	14·6	14·4	12·9	10·8	None	100	106	115	113	102
Trial no. 24† Haverton Hill	Wheat	Yield cwt./acre	Grain	31·5	33·1	33·4	32·2	33·8	2·9	Linear regression	100	105	106	102	107
Trial no. 25,‡ Haverton Hill	Wheat	Yield	Straw	52·8	52·8	53·5	55·5	54·8	12·5	None	100	100	101	105	104
		cwt./acre	Grain	13·0	13·9	13·6	13·8	15·3	16·3	None	100	107	105	106	118
Trial no. 26, Middlewich	Oats	Yield	Straw	36·2	36·4	36·1	38·2	37·2	7·9	None	100	101	100	106	103
		cwt./acre	Grain	31·9	32·7	32·9	34·0	33·3	6·3	None	100	102	103	107	104

* This trial was harvested by binder and then threshed.
† Seed rate of 20 and 30 % γ-B.H.C. treatments about 20 % higher than other treatment. This trial was combine harvested.
‡ Seed rate fell off gradually from 0–70 % γ-B.H.C. treatments; that of the latter being about 15 % lower than that of the former.

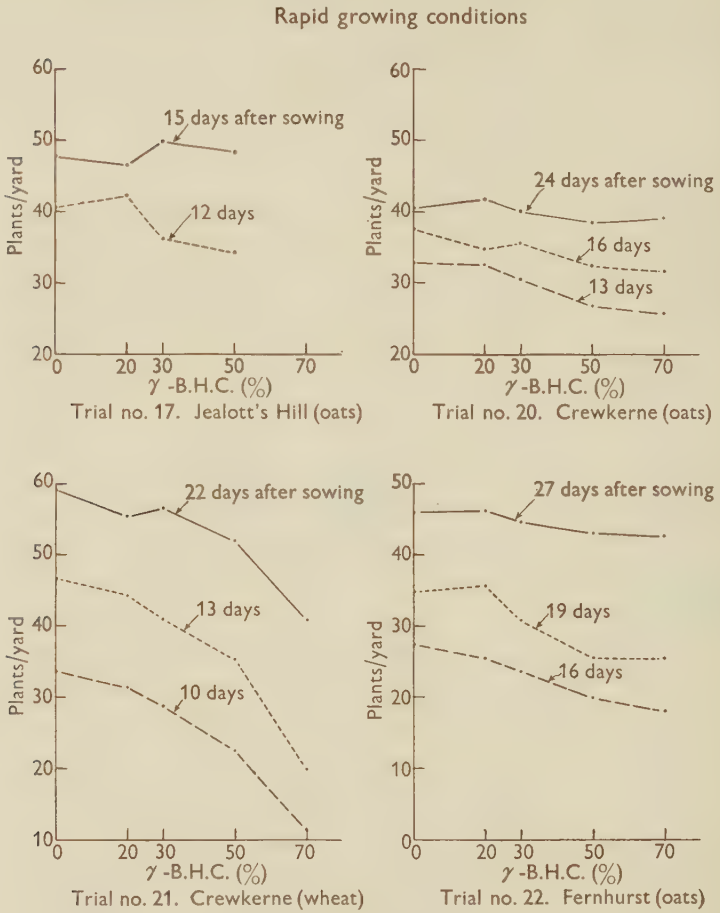


Fig. 7. Effect of γ-B.H.C. seed dressings on plant population of autumn (1947)-sown trials.

Experiments covering overdressing and effect of storing dressed seed

Overdressing

Overdressing was studied in laboratory experiments with attention to dressing load, and to contents of γ-B.H.C. and organomercurial. The γ-B.H.C. content ranged from 0–70 % at the 2 oz. rate and from 0–35 % at the 4 oz. rate. The organomercurial was 1 % at the 2 oz. rate and 0·5 or 1 % at the 4 oz. rate. Dressed seed was sown in sterilized soil and emergence counts were made daily until maximum emergence was reached.

Tables 6 and 7 record mean percentage germination on the 10th and 22nd day

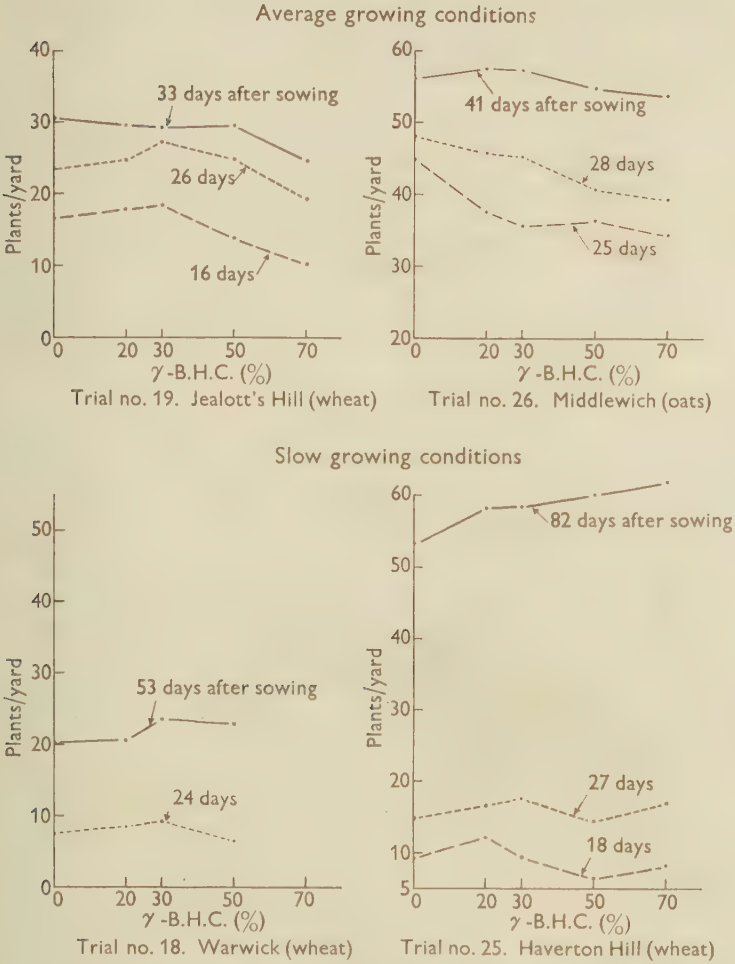


Fig. 8. Effect of γ-B.H.C. seed dressings on plant population of autumn (1947)-sown trials.

after sowing. Figures for the 10th show that delayed emergence is caused both by excess γ-B.H.C. and excess organomercurial at constant dressing rate and also by higher dressing rates at constant γ-B.H.C. and organomercurial loads. Figures for the 22nd day show no significant changes with varying γ-B.H.C. or organomercurial concentrations and no significant differences between normal and twice-normal dressing rates.

Storage

In preliminary storage tests seed wheat was treated, up to 7 oz./bushel, with

TABLE 6. *Mercury load constant at 0·02 oz./bushel: γ-B.H.C. load and dressing load variable*

Dressing load (oz./bushel)	Hg load (oz./bushel)	γ-B.H.C. % in dressing	γ-B.H.C. Load on seed (oz./bushel)	Mean % germination after 10 days	Mean % germination after 22 days
2	0·02	0	0·0	84·4	92·0
		25	0·5	87·6	96·8
		50	1·0	78·8	92·4
		70	1·4	65·2	90·8
4	0·02	0	0·0	73·6	96·4
		12·5	0·5	76·8	94·8
		25·0	1·0	71·2	89·6
		35·0	1·4	60·4	92·0

Statistical significance

	10 days	22 days
Reduction in mean % germination with increase in γ-B.H.C. load	Significant at 5 % level	Not significant
Reduction in mean % germination with increase in dressing load	Significant at 1 % level	Not significant

TABLE 7. *Dressing load constant at 4 oz./bushel: γ-B.H.C. load and mercury load variable*

Dressing load (oz./bushel)	Hg load (oz./bushel)	γ-B.H.C. % in dressing	γ-B.H.C. Load on seed (oz./bushel)	Mean % germination after 10 days	Mean % germination after 22 days
4	0·02	0	0·0	73·6	96·4
		12·5	0·5	76·8	94·8
		25	1·0	71·2	89·6
		35	1·4	60·4	92·0
	0·04	0	0·0	62·4	94·0
		12·5	0·5	66·8	95·6
		25	1·0	42·4	92·4
		35	1·4	44·0	89·6

Statistical significance

	10 days	22 days
Reduction in mean % germination with increase in γ-B.H.C. load	Significant at 1 % level	Not significant
Reduction in mean % germination with increase in mercury load	Significant at 1 % level	Not significant

a dressing containing 20 % γ-B.H.C. and stored in small, air-tight containers for 4 weeks. The treatment was without effect on total emergence.

In a further test, samples of wheat, oats and barley, of normal and high moisture content, were treated at 2 oz./bushel with dual-purpose dressings containing 0, 20 and 30 % γ-B.H.C. and 1 % mercury as organomercurial. The dressed seed was

TABLE 8. *Effect on germination of storing cereal seed dressed with γ-/Hg seed dressing*

N.B. All γ-B.H.C. levels contained 1 % Hg as organomercurial. Dressing applied at 2 oz./bushel.

(*a*) Actual data

% Germination in soil

	Wheat						Oats						Barley					
	Variety Little Joss (Moisture 15·2 %)			Variety Standard Red (Moisture 16·8 %)			Variety Victory (Moisture 13·4 %)			Variety S. 84 (Moisture 17·5 %)			Variety Plumage Archer (Moisture 13·2 %)			Variety Spratt Archer (Moisture 16·0 %)		
% γ-B.H.C.	Nil	20 %	30 %	Nil	20 %	30 %	Nil	20 %	30 %	Nil	20 %	30 %	Nil	20 %	30 %	Nil	20 %	30 %
Storage period																		
0	91·0	93·6	86·6	87·0	90·6	86·6	80·6	78·0	84·6	88·6	88·6	92·6	87·6	84·0	86·0	97·0	96·0	97·0
2 weeks	95·6	98·6	98·0	95·0	97·6	95·6	79·6	84·0	81·0	92·0	91·6	89·0	91·6	91·0	91·6	99·0	98·6	100·0
1 month	97·0	99·0	95·0	98·6	97·6	99·0	80·6	85·0	78·0	85·0	90·6	90·0	91·0	91·0	90·0	97·6	98·0	98·0
3 months	86·6*	95·6	93·0	95·0	97·0	97·0	90·0	80·0	91·0	92·0	88·0	91·0	90·6	89·6	91·0	98·6	100·0	100·0
6 months	96·6	91·6	94·6	99·6	98·0	97·0	79·0	78·0	75·6	91·0	88·6	91·0	89·6	89·0	87·0	98·6	98·6	98·6
9 months	86·0*	88·6	92·0	97·6	97·0	89·0	82·6	86·6	78·6	89·0	88·6	79·0*	73·6*	86·6	85·6	97·6	93·6	81·6
12 months	92·6	90·6	90·0	97·6	93·6	95·6	71·0	77·0	69·6	88·0	89·6	88·0	87·0	83·6	80·0	98·0	99·0	99·0

(*b*) Data calculated on basis of control (lower moisture) = 100

Storage period																		
0	100	103	95	96	100	95	100	97	105	110	110	115	100	96	98	111	110	111
2 weeks	100	103	102	99	102	100	100	105	102	115	115	112	100	99	100	108	108	109
1 month	100	102	98	102	101	102	100	105	97	105	112	112	100	100	99	107	108	108
3 months	100	110	107	110	112	112	100	89	101	102	98	101	100	99	100	109	110	110
6 months	100	95	98	103	101	100	100	99	96	115	112	115	100	99	97	110	110	110
9 months	100	103	107	113	113	103	100	105	95	108	107	96	100	118	116	133	127	111
12 months	100	98	97	105	101	103	100	108	98	124	126	124	100	96	92	113	114	114

* One of the four replicates had an anomalously low germination.

placed in small hessian sacks and stored for a year in a dry, airy, brick building. Samples were taken at intervals and submitted to germination tests in soil. Germination was not materially affected by any of the treatments, as shown by the data in Table 8.

Acknowledgement is made to the Ministry of Agriculture Advisory Entomologists (now the N.A.A.S.) in the various provinces; to the Berks Agricultural Executive Committee for assistance in finding sites and to farmers who made the sites available. For the loan of the 'Somerset' combine drill used in laying down the trials in the Hawthorndale area we must thank Messrs Dening, of Chard, Somerset.

We are grateful to our many colleagues who assisted with these trials; in particular to Mr E. C. Edgar and Mr F. C. Peacock who bore major shares. Thanks are due to Mr P. A. Collier and Mr W. D. Ewan of the Statistical Department, Jealott's Hill for analysing the data.

The 'Gammexane' γ-B.H.C., used in the preparation of the dressings, was supplied by I.C.I. (General Chemicals) Ltd., and formulation was carried out by I.C.I. (Dyestuffs) Ltd., mainly by Mr F. L. Sharp.

REFERENCE

Jameson, H. R., Thomas, F. J. D. & Woodward, R. C. (1947). The practical control of wireworm by gamma benzene hexachloride ('Gammexane'): Comparison with dichlorodiphenyltrichlorethane (D.D.T.). *Ann. appl. Biol.* **34**, 346–56.

(*Received* 15 *May* 1950)

THE EFFICIENCY OF CHEMICAL TREATMENTS OF VEGETABLE SEEDS AGAINST SEED-BORNE AND SOIL-BORNE ORGANISMS

By HARRY JACKS

*Department of Plant Pathology, Imperial College of Science and Technology, London, S.W. 7**

(With 4 Text-figures)

Out of thirty-four newer fungicides tested in greenhouse and field trials, red cuprous oxide (Cuprocide), tetrachloro*para*benzoquinone (Spergon), methyl mercury dicyandiamide (Panogen), tetramethylthiuram disulphide (T.M.T.D.), ferric dimethyldithiocarbamate (F.D.D.), zinc trichlorophenate (Dow 7B) and 1-*p*-sulphamylphenyl-3:5-dimethyl-4-nitrosopyrazole (36L) were selected for detailed investigation on lettuce, pea, French bean, turnip, red beet, tomato and celery. Derivatives of dithiocarbamic acid, and 36L and Spergon among non-metallic compounds compared favourably with cuprous oxide and organomercurial compounds. Addition of hormones, insecticides or nemacides did not improve emergence. Soil tests were more satisfactory than laboratory selection. Rates of application recommended by manufacturers often injured small seeds, and the optimum dosages were determined by extensive tests.

Results obtained depended on conditions of test. Under glass, emergence was lower at 5–12° C. than at 16–20° C. Emergence was optimum at 45 % water-holding capacity, and additional tests are required at higher and lower moisture to assess phytocidal and protectant action.

In field trials seed treatments were generally most beneficial in cool and moist soil, and improved emergence in all but driest soil. T.M.T.D., Spergon and 36L were the most effective seed protectants. Mixing of these materials did not improve emergence. Emergence in relatively dry soil (25 % W.H.C.) was improved by watering after sowing, but emergence was decreased in moist soil by watering within 2 days of sowing.

A seed-borne form of *Rhizoctonia solani* was isolated from most seeds. *Pythium*, *Rhizoctonia* and *Fusarium* were commonly isolated from seedlings. *Pythium* was commoner at low and *Fusarium* at higher temperatures. Surface disinfection of seed by Spergon and Panogen compared favourably with acidulated mercuric chloride. *Ascochyta* on pea seed was not controlled, but all treatments except Cuprocide improved emergence of spotted pea seed. T.M.T.D., 36L and Spergon were the most efficient in controlling pre-emergence damping off. Seed treatment was more beneficial for pea than other vegetables, but emergence was usually improved by protectants except for turnip and celery seed.

Treated seed stored dry for 10 months gave better emergence than untreated seed, and showed no evidence of diminished germination.

The object of this study was to examine a considerable number of newly introduced seed-protective substances and to select the most effective disinfectants and protectants by tests on vegetable seeds under a wide range of conditions.

* Now of Plant Diseases Division, Owairaka, Auckland, N.Z.

MATERIALS AND METHODS

The varieties of vegetable seed used in this work were as follows, the dates in brackets giving the origin of the seed: lettuce, Imperial (1947); pea, Laxton Superb, a wrinkled variety (1946); French bean, Canadian Wonder (1947); turnip, Early Snowball (1947); red garden beet, Dewar's Improved (1947); tomato, Early Market (1947); celery, Giant White (1945).

Methods of seed examination followed directions given by Doyer (1938) and Brett (1939), samples being taken by inserting a tube into the bulk of seed. Ten samples, each of 100 seeds, were examined under a binocular microscope (×25) and results are given in Table 1. Moisture content of seed was ascertained from the mean of four samples of each variety dried at 100° C., for 24 hr.

TABLE 1. *Quality of seed. Data* (%) *based on ten samples of* 100 *seed each*

	Lettuce	Pea	French bean	Turnip	Beet	Tomato	Celery
Discoloured	1·5	1·0	7·7	11·0	19·0	8·0	11·0
Foreign bodies	5·0	1·5	4·3	6·5	5·0	10·0	11·0
Weeds	6·0	2·0	3·0	6·0	10·0	—	9·0
Mycelium	18·0	12·0	9·0	8·0	—	13·0	5·0
Sclerotia	11·0	2·5	1·5	2·0	3·0	9·0	7·0
Ascochyta (pyc.)	—	6·0	—	—	—	—	—
Septoria (pyc.)	—	—	—	—	—	—	13·0
Insect damage	—	1·0	1·2	—	—	—	—
Mechanical damage	3·0	5·0	3·0	2·0	General	—	12·0
*Phoma**	—	—	—	—	57·0	—	—
Moisture content	7·3	12·7	13·9	6·8	10·6	6·4	9·1
Weight of 100 (g.)	0·147	28·84	36·54	0·196	1·37	0·312	0·038
Variability (%) ±	3·5	3·5	2·5	3·6	6·9	3·7	4·3

* On seedlings germinated on filter-paper

The seed used gave satisfactory germination from the commercial point of view. It will be noticed, however, that there was substantial contamination with specific parasites, viz. pea with *Ascochyta*, beet with *Phoma* and celery with *Septoria*. Doyer (1938) points out that determination of the usually high percentage infection of beet seed with *Phoma betae* has only relative value as the seedlings outgrow the effect of the fungus. Adverse conditions may, however, increase the susceptibility of seed.

According to Hulbert & Whitney (1934) and Ledingham (1946) seed injury brought about by harvesting operations results in reduction in emergence. It was claimed that cracking of the testa was seldom found in hand-harvested seed lots and emergence was consequently higher. McNew (1943) found that treatment protected injured seed from attack of organisms harmful to germination.

Altogether forty-four chemical preparations were obtained from various sources and of these seven of widely different composition were tested. The remainder were not used, mainly because they did not differ essentially from some of those under

examination. Materials selected for thorough investigation are listed in Table 2. Other materials tested included: yellow cuprous oxide, Phygon; a number of other organo-mercury preparations and calomel; various derivatives of dithiocarbamic acid; polyethylene polysulphide latex; pentachloro-dinitrobenzene; and a number of mixed preparations containing hormones, vitamins, D.D.T. and B.H.C.

TABLE 2. *List of materials used*

Trade name or code	Active ingredients (%)	Source
(1) *Cuprous oxides*		
Cuprocide	94·0 Red cuprous oxide	Rohm and Haas Co. Ltd., U.S.A.
(2) *Quinones*		
Spergon	98·0 Tetrachloro-*para*-benzoquinone	Naugatuck Chemical Co. Ltd., U.S.A.
(3) *Organo mercury compounds*		
Panogen	0·80 Methyl mercury dicyandiamide	Aktiebolat Lauxein-Casco, Sweden.
(4) *Derivatives of dithiocarbamic acid*		
T.M.T.D.	100·0 Tetramethyl thiuram disulphide	Robinson Brothers Ltd., U.K.
F.D.D.	100·0 Ferric dimethyl dithio-carbamate	Robinson Brothers Ltd., U.K.
(5) *Others*		
Dow 9 B	50·0 Zinc trichlorophenate	Dow Chemical Co. Ltd., U.S.A.
36 L	100·0 1-*p*-sulphamylphenyl-3:5-dimethyl-4-nitrosopyrazole	Naugatuck Chemical Co. Ltd., U.S.A.

Materials were applied as percentages of seed weight. Accurate dosages of dusts or liquids were weighed or pipetted and applied to small amounts of seed, even distribution being ensured by shaking both in a conical flask for 5 min. Larger amounts were treated in a 2 lb. jar fitted into a churn, which was made from parts of a Meccano set on similar principles to those described by Fitzgibbon (1943). Churning in alternate directions for 5 min. ensured even coating of seed. Small seed was placed in a suitable container fitted into the jar. The rate of application was ascertained by re-weighing treated seed and continuing treatment until the desired dosage was obtained. Small dosages of dusts were applied in dilution with talc, those of liquids with water.

The maximum adherent capacity of selected materials was ascertained for purposes of testing the effect of progressive dosages. In these determinations, the chemical substances were applied in excess to a weighed amount of seed, viz. to 20 g. of pea or bean, 10 g. of beet and 2 g. of lettuce, turnip, tomato and celery. After mixing for 5–10 min. seed was dropped from a height of approximately 12 in. on to a fine sieve (80 × 1·0 mm. × 4 in. diam.) when non-adhering material was screened off. The percentage which adhered was then assessed by re-weighing.

In the case of some substances which showed poor adherence to the seed surface, a preliminary treatment with a wetting agent was used. This was prepared by dissolving 3 g. resin in 10 ml. methylated ethyl alcohol and was used at the rate of 0·25 % of seed weight.

Dispersible materials were not as adherent as non-dispersible and required longer mixing to ensure even coating of seed. Higher dosages of dispersible materials were

applied by the short wet method of Muskett (1944). Although a number of these materials are poisonous, none except Dow 9B caused inconvenience during application.

Soil of a medium sandy loam was used in all laboratory and greenhouse tests. Analysis of this soil and of samples taken from the area allotted for field trials was almost identical. The analysis was as follows: potash (K_2O) 5 mg./100 g., phosphate (P_2O_5) 20 mg./100 g., organic content 6·8 % and pH measured by glass electrode meter 6·45. Before use the soil was air-dried and sieved, and its water holding capacity (W.H.C.), viz. 56 %, was ascertained by the standard method of Keen & Raczkowski (1921) as modified by Garrett (1946). Lower moisture levels, i.e. 25 and 45 % saturation, were adjusted before potting, by adding calculated amounts of water to a weighed quantity of soil. Uniformity was obtained by mixing soil and water first by hand and finally passing through a 4 mm. sieve. The highest level of soil moisture, i.e. 65 % saturation, was obtained by adding measured amounts of water to soil already potted.

Sterilized soil was obtained by autoclaving for 2 hr. at 25 lb. pressure; and subsequently air-dried and stored for 14–20 days before use.

Circular tins (12·5 cm. diam. and 10 cm. deep), protected from rust by a thin film of bituminous paint and each holding 1 kg. soil, were used in greenhouse tests. Before sowing, approximately 100 g. of soil for small, or 200 g. for large seed was removed from each tin. The surface of the remaining soil was lightly tamped down, fifty small or twenty-five large seed were sown in concentric circles and the soil previously removed was replaced and levelled, giving a surface 3 cm. below the rims of the tins. This method gave a depth of sowing of 1·2 cm. for small, 2 cm. for beet and 3 cm. for large seed.

Each trial was replicated four times and tins were placed on benches in a randomized order (Youden, 1940), the greenhouse temperature ranging from 15 to 18° C. Low temperature tests were carried out by keeping the tins for 7 days at 5° C. and removing them to a cool room at 8–12° C. Tins were covered with glass squares to avoid loss of moisture and these were removed when the seedlings grew tall enough to reach them. Weight of tins was checked on alternate days. No loss of moisture was recorded until removal of glass covers, deficiencies being made up afterwards by sprinkling water over the soil surface.

Seedlings were dug out 15–30 days after emergence, counted and examined for lesions, and separate records were made for healthy and diseased seedlings. This gave a better judgement of the effect of treatments than did records of total emergence. Counts of diseased plants included some with only very slight lesions. These might have survived and grown into plants similar to those derived from healthy seedlings. Results, however, suggest that treatments, although not giving protection against post-emergence damage, appear to bring about increased vigour enabling a greater number of seedlings to survive pre- and to resist post-emergence attack.

Isolations from ungerminated seed and from abnormal or diseased seedlings were made by the methods described by Baylis (1941). Unless otherwise stated 100 diseased seedlings taken at random from all treatments of a particular experiment were so used.

Field trials were carried out at the Imperial College Biological Field Station at Slough. Dates of sowing were spaced, so as to permit observation of the effect of environmental conditions on activity of soil-borne organisms and on protectant action of treatments. Seed was treated no longer than 2–4 days before sowing. Envelopes containing fifty pea or bean or equal weights of smaller seed were prepared for each sowing. For smaller seed, four lots of 300 seed each were counted and weighed, averaging 0·42 g. for lettuce, 0·57 g. for turnip and 4·1 g. for beet.

Trials consisted of blocks composed of a number of 9 ft. rod row treatments randomized within each block and replicated five times in accordance with recognized field plot procedure (Wishart, 1940). Drills were drawn at a depth of 4 cm. for pea or bean seed, 3 cm. for beet and 1·5 cm. for lettuce and turnip. Atmospheric and soil temperatures at seed level were recorded twice daily. A daily record of precipitation was kept and soil moisture expressed in percentages of total w.h.c. was assessed twice weekly. Date of emergence was noted and final records were taken in a manner already described by lifting all plants after sufficient time had elapsed for maximum emergence to take place. An estimation of time required for maximum emergence at different temperatures and soil moistures had been ascertained.

Results were subjected to analysis of variance. Germination percentages in sets of data containing values below 20 % or above 80 % were transformed to corresponding angular values before analysis (Fisher & Yates, 1948). Data containing values of 0·0 % were transformed to $\sqrt{(x+\frac{1}{2})}$ for analysis. For clearer interpretation of results, some tables containing both actual and transformed values have been simplified by giving only values subjected to analysis. Significant differences at 5 % level, higher than controls for healthy seedlings and lower than controls for diseased seedlings, are indicated by italic figures in the tables unless otherwise stated.

Other methods are more conveniently given in the respective chapters of the text.

Viability tests and speed of emergence

For testing the viability of vegetable seed, sowing on moist sand proved to be the most suitable method. The optimum temperatures for germination were as follows: lettuce 20–25° C.; pea and turnip 20–30° C.; French bean and tomato 25° C.; beet 25–30° C.; and celery 15–18° C. Speed of emergence increased with temperature and soil moisture. Emergence was higher in unsterilized than in autoclaved soil, but depth of sowing was not of significant importance provided the normal limits are observed. There is a slight reduction in the speed of emergence in soil inoculated with *Pythium* sp. Some materials, e.g. Cuprocide, Dow 9B, etc., may reduce speed of emergence, especially under dry soil conditions.

Tests were also carried out to ascertain the minimum period before trials could

safely be recorded under field conditions and it was found that at low temperatures (5° C.) lettuce could be recorded after 12 days, pea after 20 and turnip after 24 days. At medium temperatures (12° C.) lettuce could be recorded after 6 days, pea after 14, French bean after 18, turnip after 12 and beet after 30 days. At higher temperatures (20° C.) emergence was accelerated and lettuce could be recorded after 2 days.

Preliminary selection of seed protectants

Preliminary greenhouse and field trials were made to obtain a tentative selection of the more promising materials. A number of subsidiary laboratory experiments were also made but these were not considered to be reliable, so that they will be dealt with in summary form only.

Fungicidal tests *in vitro* were carried out by measuring the effect of various doses of the preparations on germination of spores of *Alternaria solani* as measured by the haemocytometer method. It was possible by these means to differentiate between various preparations; thus Perenox C and P.C.D.B. inhibited germination at 100 p.p.m., Phygon at 50 p.p.m., T.M.T.D. (50 % D) and F.D.D. at 25 p.p.m. and Panogen at 12·5 p.p.m. The method was not pursued, largely because it was not obvious that the results bore any direct relationship to the behaviour of the substances as seed protectants in soil.

A second laboratory method was to inoculate plates of medium (malt extract agar and soil extract agar, as representing high and low nutritive levels respectively) with various fungi, so that young circular colonies were established. Eight seed, with or without protectant, were then planted a short distance ahead of the advancing culture. From the manner of growth of the cultures one could then judge how far the protective substances were acting fungistatically.

In the absence of protectants, the seed became overgrown by the fungi, and in some cases, e.g. lettuce seed with a culture of *Botrytis cinerea*, the seed was killed before it could germinate. In the presence of a protectant the seed might be overgrown but nevertheless germinate. The effects of the protectants on fungal growth were more pronounced on the weak than on the rich medium. Tested in this way, certain well-known fungicides give no protection against *Botrytis cinerea*.

Though various workers have claimed to assess the value of plant protective substances by methods of this type, it is clear that the conditions are far removed from those prevailing in practice, and in fact some of the substances which gave very unpromising results in these tests (e.g. Spergon) proved to be most useful seed protectants when tested in soil. Little value therefore is to be attached to this method.

As a third laboratory test, the Ulster method as described by Muskett & Malone (1941) was examined in some detail and shown to be the most satisfactory of the laboratory methods. It is, however, subject to serious error. All the copper preparations tested were ineffective in checking fungal growth, and would thus be discarded on the basis of this test, whereas in fact some of them are valuable as seed protectants (Croxall & Ogilvie, 1942; Baylis, Deshpande & Storey, 1943; Sidky,

1947; and Brown & Montgomery, 1948). Furthermore, it was found that certain preparations of tetramethyl thiuram disulphide were equally effective as tested by the Ulster method whereas in the more practical tests in soil, they showed considerable differences in efficiency. Glasshouse and field tests were relied upon therefore for selecting protectants for further study.

An experiment was carried out in a greenhouse (16·5° C. ± 2·5) with twenty-eight preparations, in soil maintained at 25, 45 and 65 % W.H.C. Table 3 gives the mean values for the three water contents used of the six preparations selected for further test; significant differences from the control are shown by italics.

TABLE 3. *Mean percentage emergence from three moisture levels; also effect of seed treatments on average height and weight*

	Lettuce				Pea			
	Emergence		Height	Weight	Emergence		Height	Weight
Treatment	H.	D.	(cm.)	(g.)	H.	D.	(cm.)	(g.)
Control	52·3	21·3	4·0	0·03	36·8	15·5	8·5	0·8
Cuprocide	*63·9*	*3·2*	*4·2*	0·03	*66·2*	*3·7*	*8·2*	0·8
Spergon	*65·9*	*2·1*	*4·4*	0·03	*80·9*	*1·2*	*10·6*	*1·0*
F.D.D.	*77·5*	*1·8*	4·0	0·03	*71·4*	*8·5*	*10·2*	*1·1*
T.M.T.D.	*71·1*	0·3	4·0	0·03	*88·2*	*2·1*	*10·0*	*1·2*
Panogen	*65·9*	*2·2*	*4·3*	0·03	*66·2*	9·7	*9·5*	*1·0*
Dow 9 B	*65·5*	*1·3*	3·5	*0·04*	*44·0*	*24·6*	8·1	*1·0*
Signif. at 5 %	—	—	0·23	0·0015	—	—	0·9	0·08

H. = healthy. D. = diseased.

The seed protectants were applied at the rate of 2 % of seed weight for lettuce (except Panogen applied at 0·5 %); and at 0·25 % for pea seed.

It will be noted that more substances were effective in reducing the percentage of diseased seedlings emerging than in increasing the percentage of healthy seedlings.

Table 4 summarizes the results of the six best fungicides in a field test with thirteen preparations carried out in the early spring of 1948. The average daily temperature was 14·9° C. (± 1·3°) and soil moisture 43·7 % (± 2·7 %).

TABLE 4. *Emergence percentage in a preliminary field trial*

	Lettuce			Pea		
Treatment	H.	D.	Weight (g.)	H.	D.	Weight (g.)
Control	38·6	14·6	0·10	53·6	16·2	2·3
Cuprocide	*55·0*	*5·4*	0·09	*72·4*	*7·9*	1·7
Spergon	*58·6*	*3·8*	0·10	*73·2*	6·6	*2·7*
F.D.D.	*49·7*	*6·0*	0·09	*74·8*	*12·2*	2·3
T.M.T.D.	*52·7*	*6·2*	*0·11*	*78·0*	*6·2*	2·3
Panogen	*56·4*	*4·5*	0·10	*73·4*	*8·8*	*2·4*
Dow 9 B	38·9	*2·6*	*0·11*	*68·8*	*8·0*	*2·7*
Signif. at 5 %	4·5	4·1	0·002	14·4	5·4	0·05

Six materials, Cuprocide, Spergon, Panogen, T.M.T.D., F.D.D. and Dow 9B were chosen as a result of these tests as the main seed protectant substances for further work. Dow 9B, which was non-effective on lettuce but effective on pea, was included because it is a different type of compound from the remainder. One preparation, 361, was not obtained in time for the earlier tests, but was included with the six already selected in some of the later tests.

Dosage tests of selected seed protectants

The standard recommendations of manufacturers for dosages of seed protectants are as follows: 2 % of the seed weight for small seed (lettuce, turnip, beet, tomato, celery, etc.), and 0·25 % for large seed (pea and bean). The effect of a range of dosages was tested in soil, both under greenhouse and open-field conditions. In the greenhouse experiments the temperature was 16·9° C. (±3·4°) and soil moisture was maintained at 45 % W.H.C.

Tables 5, 6 and 7 give the results with the same notation for significance as before. Tests with 361 were carried out later and data for this substance together with their comparative controls have been added to the tables.

Some materials (viz. Panogen) showed considerable phytocidal damage on lettuce at the 2 % rate, and others (viz. Cuprocide, Spergon and T.M.T.D.) at the same dosage gave significantly lower emergence than at 1 %. Other seeds were not as sensitive as lettuce and results suggest that, provided care is taken not to exceed certain limits, careful dosage estimation is necessary only for such seeds as are known to be sensitive to overdose with certain substances. Graphic presentation by dosage response curves (Albert, Horsfall, Heuberger & Stoddard, 1941) gave slightly sloped portions of curves for the lowest and highest dosages of some materials, Panogen alone showing pronounced variations at the highest dosage. The remainder of the curves were flat and this confirms results obtained by McCallan (1947) on pea and Clayton (1948) on okra.

Whereas lettuce, pea and bean responded well to treatments, turnip showed almost no response, and others showed higher sensitivity towards the various protectants (viz. beet giving increased emergence after treatment with Panogen and T.M.T.D., tomato not responding to Cuprocide, and celery responding only to Cuprocide and F.D.D.).

Records of plant growth taken from all dosages showed little variation except where phytocidal effect was significant.

The effect of dosage levels on emergence of lettuce and pea was also tested in the field and results are given in Table 8.

The same tendencies as in greenhouse tests were observed, with the exception of increased response to the lowest dosages applied to pea.

In some cases *ad lib.* addition of protectants is recommended by manufacturers, viz. dusts heaped on the tip of a penknife and added to small quantities of seed,

TABLE 5. *Effect of various dosages on emergence of lettuce, turnip and beet*

	Lettuce								Turnip								Beet							
	0·25 %		0·5 %		1·0 %		2·0 %		0·25 %		0·5 %		1·0 %		2·0 %		0·25 %		0·5 %		1·0 %		2·0 %	
Treatment	H.	D.	H	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.
Control	—	—	—	—	60·6	19·7	—	—	—	—	—	—	73·7	16·3	—	—	—	—	—	—	59·0	25·0	—	—
Cuprocide	80·8	8·0	95·5	0·0	80·0	1·2	67·4	1·9	66·5	1·4	75·8	2·4	66·5	1·1	69·8	6·8	48·3	18·0	68·8	15·5	49·0	21·0	52·5	11·3
Spergon	88·6	2·2	94·0	0·0	82·0	0·0	63·4	3·1	71·2	5·8	79·3	2·6	84·3	0·0	81·0	0·0	36·8	15·3	42·8	21·5	32·5	26·3	37·5	23·0
Panogen	69·1	2·0	97·3	2·1	89·4	1·9	14·2	68·1	65·0	13·4	71·8	11·3	72·0	0·8	77·3	0·1	52·8	15·3	81·0	11·5	70·0	2·3	80·3	1·8
T.M.T.D.	82·2	6·4	89·7	0·8	95·3	0·0	67·5	0·5	76·3	1·3	89·0	1·3	78·3	0·0	73·3	0·0	57·3	16·0	52·0	17·8	75·5	2·0	78·8	0·0
F.D.D.	84·0	0·0	75·0	0·0	83·3	0·0	78·0	2·9	67·0	13·7	66·3	19·6	75·0	5·2	70·0	0·0	50·3	18·5	43·3	27·8	65·8	8·8	64·8	8·0
Dow 9 B	70·0	12·0	92·7	2·1	94·0	1·5	85·8	0·8	57·0	8·3	83·0	2·4	80·5	1·4	66·8	1·0	60·3	13·0	82·5	7·8	65·8	3·5	68·8	0·0
Control	—	—	—	—	71·8	9·5	—	—	—	—	—	—	65·3	14·8	—	—	—	—	—	—	60·3	24·8	—	—
36 L	87·0	2·0	87·0	2·0	88·0	0·0	83·0	0·0	95·0	0·0	92·0	0·0	82·0	4·5	87·0	4·0	75·0	21·0	89·0	6·0	116·0	13·0	86·0	13·0

TABLE 6. *Effect of various dosages on emergence of pea and bean*

	Pea										French bean									
	0·063 %		0·125 %		0·25 %		0·5 %		1·0 %		0·063 %		0·125 %		0·25 %		0·5 %		1·0 %	
Treatment	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.
Control	—	—	—	—	37·4	28·6	—	—	—	—	—	—	—	—	35·0	49·0	—	—	—	—
Cuprocide	49·3	25·3	62·7	11·1	66·8	7·0	83·6	3·7	89·1	3·4	69·3	5·3	87·5	7·0	81·3	8·8	81·0	12·5	81·6	13·7
Spergon	48·7	19·3	55·9	15·4	76·9	8·1	79·2	5·9	75·3	7·4	65·3	4·0	86·4	9·3	97·0	2·0	98·0	2·0	100·0	0·0
Panogen	20·3	30·2	44·7	39·3	68·3	7·6	78·4	4·4	82·6	6·6	80·0	4·0	82·6	16·0	97·3	1·3	97·5	2·0	98·0	1·1
T.M.T.D.	58·0	14·0	83·6	6·8	84·9	4·4	80·9	6·9	87·4	7·4	62·7	5·3	91·8	7·3	88·2	9·8	94·0	6·0	98·0	2·0
F.D.D.	29·7	27·7	27·2	48·8	32·5	43·6	71·2	8·1	85·2	5·8	49·3	6·7	78·3	15·7	88·0	12·0	98·8	1·3	100·0	0·0
Dow 9 B	26·0	23·3	33·4	34·1	31·2	29·5	89·0	2·5	88·9	1·6	52·0	2·7	97·5	2·5	96·0	2·1	99·0	0·0	91·5	0·0
Control	—	—	—	—	66·3	14·8	—	—	—	—	—	—	—	—	41·5	37·3	—	—	—	—
36 L	85·0	8·0	85·0	4·0	83·0	3·8	82·0	4·0	84·0	0·0	61·5	20·0	79·0	7·0	86·0	3·5	89·0	3·3	61·0	14·0

TABLE 7. *Effect of various dosages on emergence of tomato and celery*

	Tomato								Celery							
	0·25 %		0·5 %		1·0 %		2·0 %		0·25 %		0·5 %		1·0 %		2·0 %	
Treatment	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.
Control	—	—	—	—	49·5	40·5	—	—	—	—	—	—	23·8	6·9	—	—
Cuprocide	50·8	*20·3*	55·0	*19·1*	58·5	*19·1*	62·3	*15·9*	26·0	5·9	31·8	*0·5*	*40·0*	*0·0*	26·5	*0·0*
Spergon	42·3	33·1	58·5	30·2	*78·5*	*27·2*	*73·8*	*11·1*	26·3	12·3	25·8	16·6	28·5	4·0	26·8	7·7
Panogen	*77·3*	*6·4*	*82·0*	*5·8*	*82·5*	*4·0*	*88·5*	*4·5*	18·0	14·5	17·5	14·1	23·3	*3·3*	13·8	4·0
T.M.T.D.	62·5	42·6	65·5	31·8	*81·3*	*1·7*	*73·8*	*0·0*	17·8	19·7	20·0	11·9	18·8	*0·0*	17·0	*0·0*
F.D.D.	63·0	37·3	*69·8*	*26·9*	*82·3*	*6·1*	*86·3*	*1·8*	*39·8*	*2·5*	*46·5*	*7·3*	*46·0*	*0·0*	*38·5*	*0·0*
Dow 9 B	60·3	36·4	*82·5*	*12·6*	*88·3*	*9·3*	*83·8*	*6·1*	26·8	3·5	33·3	*0·0*	23·8	*0·0*	24·8	*0·0*
Control	—	—	—	—	61·3	27·3	—	—	—	—	—	—	20·5	7·5	—	—
36 L	*87·0*	*11·3*	*81·0*	*6·0*	*90·0*	*2·0*	*91·0*	*0·0*	18·0	6·0	28·0	*1·0*	18·0	*0·0*	20·0	*0·0*

TABLE 8. *Effect of various dosages on emergence of lettuce and pea under open field conditions*

Dosage	Cuprocide		Spergon		Panogen		T.M.T.D.		F.D.D.		Dow 9 B		36 L	
% weight	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.
Lettuce:														
Control	38·0	7·3	38·0	7·3	38·0	7·3	38·0	7·3	38·0	7·3	38·0	7·3	50·3	9·0
0·25	*60·3*	*4·0*	44·0	7·3	41·0	*4·0*	52·0	*5·0*	*56·0*	*3·7*	36·7	7·0	54·7	*1·7*
0·5	*70·0*	*2·3*	*70·3*	*2·3*	*68·3*	*3·3*	*72·3*	*0·3*	*67·7*	*4·3*	*67·3*	*1·7*	*59·8*	*3·3*
1·0	*68·0*	*2·0*	*71·0*	*2·3*	*63·0*	*1·0*	*69·7*	*0·7*	*55·3*	*2·0*	*58·7*	*1·0*	*63·0*	*1·0*
2·0	*62·0*	*1·0*	*56·3*	*0·0*	*53·0*	*2·0*	*59·0*	*1·0*	*67·0*	*1·0*	*59·0*	*1·0*	*67·0*	*2·0*
Pea:														
Control	33·4	12·0	33·4	12·0	33·4	12·0	33·4	12·0	33·4	12·0	33·4	12·0	40·7	13·3
0·063	*54·7*	8·0	*65·3*	*6·7*	*61·3*	*5·3*	*70·7*	*2·7*	*57·3*	*2·7*	*52·0*	8·0	*77·3*	*4·0*
0·125	*72·0*	*1·3*	*64·0*	*3·3*	*68·0*	*5·3*	*85·3*	*2·7*	*72·0*	*2·7*	*65·3*	*5·3*	*70·7*	*5·3*
0·25	*74·7*	*4·7*	*78·7*	*1·3*	*76·0*	*1·3*	*72·1*	*1·3*	*74·7*	*2·7*	*64·0*	*5·3*	*70·7*	*1·3*
0·5	*72·0*	*1·3*	*84·0*	*2·7*	*81·3*	*1·3*	*80·0*	*1·3*	*76·0*	*3·0*	*74·7*	*4·0*	*69·3*	*8·0*
1·0	*64·0*	*2·7*	*62·7*	*2·7*	*69·3*	8·0	*66·7*	*1·3*	*68·0*	*2·7*	*72·1*	*5·3*	*74·7*	*6·7*

Signif. at 5 %: Treatments other than 36 L. Lettuce: H: 17·3; D: 2·0.
Pea: H: 8·6; D: 4·1
36 L: Lettuce and Pea: H: 9·3; D: 3·0.

Treatments other than 36 L. Soil temp. 5·8° C. (±4·7°). Moisture 43·2 % (±2·9 %)
36 L treatment. Soil temp. 11·2° C. (±4·6°). Moisture 42·2 %(±3·7 %).

2–4 oz. per bushel of large seeds, etc. Materials were added in excess to seed, maximum load was assessed, and germination was tested on filter-paper.

Some seeds showed higher sensitivity to overdose with certain substances than others, viz. bean to Dow 9B; turnip, beet and celery to Panogen and Dow 9B; and tomato to Spergon, Panogen, T.M.T.D., F.D.D. and Dow 9B. In a number of instances (Tables 5 and 6) dosages of 1–2 % in soil tests reduced emergence and it can be assumed that the effect of higher dosages may be more pronounced in soil than on filter-paper. This opinion is borne out by the high number of abnormal seedlings showing deformed and discoloured radicles and whose survival was doubt-

ful. The high concentration of chemicals which diffused through the substrate caused obvious retarding action on the development of survivors and this may also be assumed to have greater effect under natural conditions especially on the emergence of plants with smaller seeds.

The lowest dosages which gave a significant increase of emergence over the controls, and which were not significantly less effective than the dosage which gave best emergence were as far as possible selected for further tests. All such dosages are italicized in Table 9. In some cases, e.g. turnip, where no significant increases over the controls were recorded, an intermediate dosage between the highest and the lowest was selected. These somewhat arbitrary dosages are not italicized in Table 9.

TABLE 9. *Dosages selected for further tests*

	Seed weight (%)						
Material	Lettuce	Pea	Bean	Turnip	Beet	Tomato	Celery
Cuprocide	*0·5*	*0·25*	*0·125*	0·5	0·5	0·5	*1·0*
Spergon	*0·5*	*0·25*	*0·25*	0·5	0·5	*1·0*	0·5
Panogen	*0·5*	*0·25*	*0·25*	0·5	*0·5*	*2·0*	1·0
T.M.T.D.	*0·5*	*0·125*	*0·125*	*0·5*	*1·0*	*1·0*	0·5
F.D.D.	*0·25*	*0·5*	*0·5*	0·5	1·0	*1·0*	*0·5*
Dow 9 B	*0·5*	*0·5*	*0·125*	0·5	*0·5*	*1·0*	0·5
36 L	*0·5*	*0·063*	*0·125*	*0·25*	*0·5*	*0·25*	0·5

SEED PROTECTION

Under partially controlled conditions

According to L. K. Jones (1931), Hull (1937), Baylis (1941), Sidky (1947) and others, higher soil moisture accentuated pre-emergence damping-off, thus reducing stand. Effects of temperature were not defined by these authors. McNew (1943) and others found that low temperature decreased whilst Jones, Johnson & Dickson (1926), Ledingham (1946) and others reported a correlation of seed decay with increase in temperature.

Greenhouse tests were carried out as described above with all seven seeds at three

TABLE 10. *Effect of seed treatments on percentage healthy and diseased seedlings*

	Lettuce		Pea		Bean		Turnip		Beet*		Tomato		Celery	
Treatment	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.
Control	62·4	13·8	51·7	25·2	55·4	23·6	47·0	8·8	42·3	11·6	48·8	16·5	27·0	
Cuprocide	*75·6*	*4·7*	*72·9*	*7·4*	*83·4*	*12·0*	*42·3*	*3·8*	*64·1*	*9·1*	*46·2*	*11·7*	*26·4*	
Spergon	*83·0*	*1·1*	*85·2*	*3·1*	*88·8*	*6·9*	*56·4*	*8·2*	*47·2*	*12·5*	*50·6*	*2·0*	*25·8*	
Panogen	*73·2*	*1·0*	*72·6*	*10·1*	*90·2*	*5·1*	*54·3*	*3·0*	*67·6*	*1·0*	*63·9*	*1·8*	*25·6*	
T.M.T.D.	*83·2*	*0·7*	*88·3*	*3·6*	*91·4*	*4·3*	*62·2*	*1·0*	*68·8*	*1·5*	*65·3*	*1·0*	*30·1*	—
F.D.D.	*73·4*	*4·7*	*71·2*	*12·1*	*86·9*	*1·7*	*45·5*	*5·5*	*52·8*	*10·4*	*61·2*	*6·6*	*27·8*	—
Dow 9 B	*76·3*	*4·1*	*65·0*	*13·6*	*83·6*	*8·9*	*39·4*	*4·9*	*60·9*	*0·5*	*60·3*	*1·0*	*30·2*	—
36 L	*85·1*	*1·0*	*86·3*	*4·9*	*89·2*	*6·0*	*53·5*	*3·2*	*63·3*	*2·2*	*78·9*	*0·5*	*32·9*	—

* Numbers from fifty seed balls.

moisture and two temperature levels, with the exception of tomato and celery which were tested at the higher temperature only. Table 10 shows the mean effect of seed treatments for all levels of moisture and temperature.

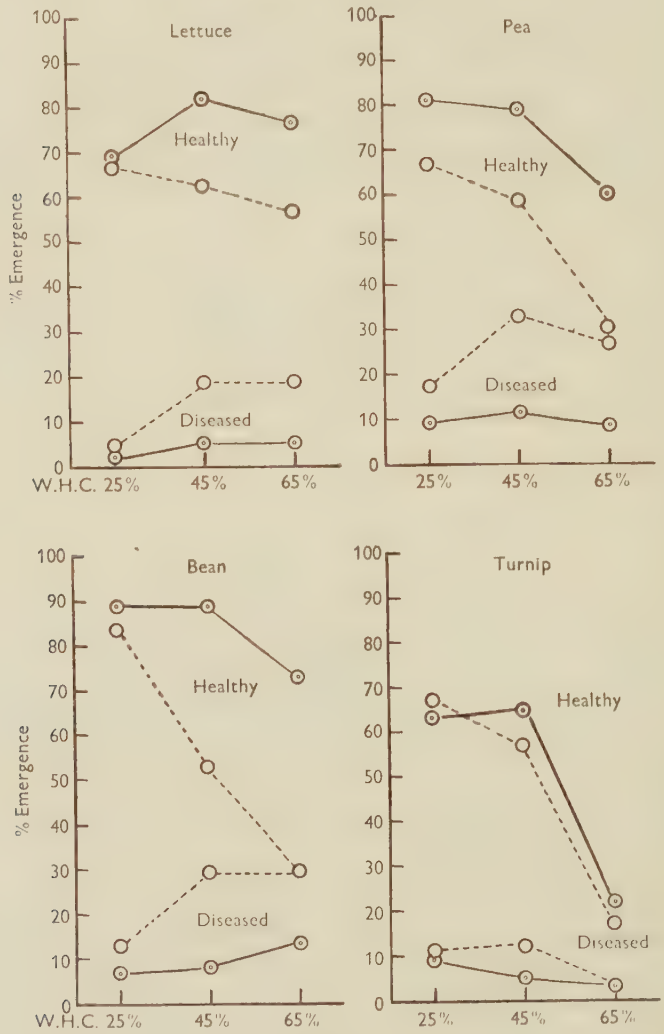


Fig. 1

Whereas total emergence was only slightly affected by treatments, separation of the data into records of healthy and diseased seedlings showed that treatments clearly increased the number of healthy and decreased the number of diseased

seedlings (Figs. 1 and 2). Percentages of diseased seedlings from different seed treatments did not vary to any extent.

Some treatments showed a certain amount of selectivity in their effect on different

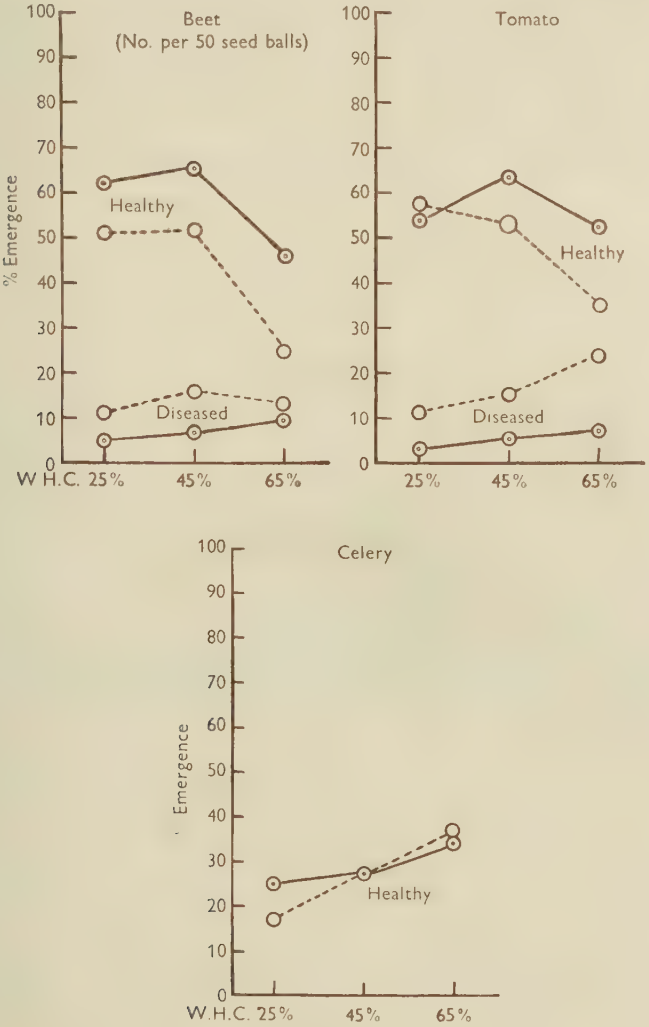


Fig. 2

plants. Thus Cuprocide and Panogen reduced emergence of lettuce in dry soil. Cuprocide and Dow 9B were toxic to turnip seed, whilst Spergon showed a similar effect on beet. Cuprocide and Spergon were not effective in protecting tomato. No

material except 36L increased emergence of celery. T.M.T.D. gave consistent results for all seeds.

The mean effect of moisture is given in Table 11, the mean being that of all treatments and the control.

It is clear from Table 11 that the general effect of dry soil is a slight reduction of emergence, and with some seeds (e.g. pea and bean) there is no reduction at all. High soil moisture (except for celery) clearly reduces emergence, most strikingly in the case of turnip.

TABLE 11. *Effect of soil moisture on percentage healthy and diseased seedlings*

W.H.C. (%)	Lettuce		Pea		Bean		Turnip	
	H.	D.	H.	D.	H.	D.	H.	D.
25	69·3	1·7	80·9	9·8	89·3	7·2	63·0	8·6
45	82·3	5·0	78·8	10·9	88·3	8·0	65·0	4·4
65	71·9	5·0	62·9	9·6	73·2	13·0	22·3	2·4

W.H.C. (%)	Beet		Tomato		Celery	
	H.	D.	H.	D.	H.	D.
25	62·0	5·1	54·0	3·2	25·4	—
45	66·0	6·3	63·4	5·4	27·4	—
65	46·0	8·1	54·0	6·8	31·9	—

Table 11 gives the combined results of all treatments, including controls. It does not show the effect of soil moisture on the behaviour of treated *versus* control. This is illustrated in Fig. 1 and 2 for all the species tested. The general effect is that high soil moisture increases the percentage of diseased and decreases the percentage of healthy seedlings, and that seed treatments counteract these tendencies.

The mean effect of temperature is shown in Table 12, the mean being that of all treatments and the control.

TABLE 12. *Effect of temperature on percentage and healthy diseased seedlings*

Temp. (° C.)	Lettuce		Pea		Bean		Turnip	
	H.	D.	H.	D.	H.	D.	H.	D.
5–12°	71·3	2·7	70·8	8·6	83·5	8·0	43·8	4·0
16–20°	77·6	5·0	77·5	11·5	83·7	10·8	56·3	5·6

Temp. (° C.)	Beet		Tomato		Celery	
	H.	D.	H.	D.	H.	D.
5–12°	51·3	6·9	—	—	—	—
16–20°	64·6	6·1	56·5	5·2	28·2	—

The two levels of temperature under test were not widely different but there was a general depression of emergence at the lower temperature range. A study of the full data showed that this effect is more pronounced with untreated than with

treated seed. The suggestion therefore is that for the evaluation of seed protectants, the tests should be carried out at low temperature levels as thereby affording a more stringent test of the efficiency of the seed protectants.

The amount of phytocidal action, if any, was examined in a test under greenhouse conditions (16–20° C.) with autoclaved soil at three moisture levels. Speed of emergence was assessed by the Kotowski formula. Table 13 gives the mean results for the three moisture levels.

TABLE 13. *Mean effect of treatments on percentage and speed of emergence*

	Control	Cuprocide	Spergon	Panogen	T.M.T.D.	F.D.D.	Dow 9B	Dow 36L
				Lettuce				
Emergence (%)	82·7	79·7	88·7	72·3	91·7	79·3	74·7	90·0
Kotowski coeff.	18·1	18·3	19·2	21·1	19·1	20·2	19·8	20·3
				Pea				
Emergence (%)	90·3	84·3	94·7	85·7	98·3	95·0	85·3	92·3
Kotowski coeff.	18·6	17·2	20·9	20·9	19·6	19·9	17·6	19·5

All treatments increased speed of emergence of lettuce, some only slightly. Apart from Cuprocide and Dow 9B the same applies for pea. Examination of the full data showed that treatments in dry soil frequently retarded emergence (especially of pea), so that the all-over increase already noted emphasizes the accelerating effect given by treatments at medium and high soil moistures.

In the open field

Sowings of pea and lettuce were made in plots at Slough at monthly intervals over the period December 1947 to March 1949, seventeen sowings in all. Seven monthly sowings were made of French bean and beet, and six of turnip, between spring and autumn 1948. During the whole period records were kept of daily rainfall and soil temperature, and soil moisture determinations were made twice weekly. Table 14 gives the mean results of all the determinations made over the period of experiment.

TABLE 14. *Mean effect of treatments over all sowings*

	Lettuce (17)*		Pea (17)*		French bean (7)*		Turnip (6)*		Beet (7)†	
Treatment	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.
Control	45·3	10·9	51·5	13·3	52·4	27·5	45·6	3·6	225·1	7·6
Cuprocide	*50·8*	*8·0*	*58·0*	*11·4*	*57·7*	*23·7*	43·6	*3·1*	*277·6*	7·6
Spergon	*58·6*	*6·0*	*73·0*	*8·3*	*70·8*	*20·7*	48·6	*3·2*	242·3	*7·1*
Panogen	*49·9*	*7·4*	*63·7*	*10·5*	*70·5*	*21·8*	45·1	*3·5*	*292·9*	*4·4*
T.M.T.D.	*62·0*	*5·4*	*75·0*	*8·2*	*76·3*	*19·7*	*52·0*	*2·9*	*289·3*	*4·4*
F.D.D.	*52·1*	*7·2*	*64·0*	*9·2*	*66·3*	*24·6*	*50·6*	*2·7*	*272·1*	*5·5*
Dow 9 B	*49·0*	*6·8*	*59·0*	*10·5*	*71·1*	*23·2*	47·9	*2·8*	*277·3*	*4·0*
36 L	*65·6*	*5·6*	*71·8*	*8·1*	—	—	—	—	—	—
Signif. at 5 %	2·9	0·7	3·0	1·3	4·2	2·1	3·8	0·5	42·7	1·0

* Number of sowings. † Numbers from 300 seed balls.

All treatments were effective with lettuce, pea and French bean; beet responded to all except Spergon; the effect on turnip was comparatively slight. The most effective treatments were T.M.T.D., Spergon and 36L (so far as the latter was tested). Cuprocide was phytocidal to bean at low temperatures.

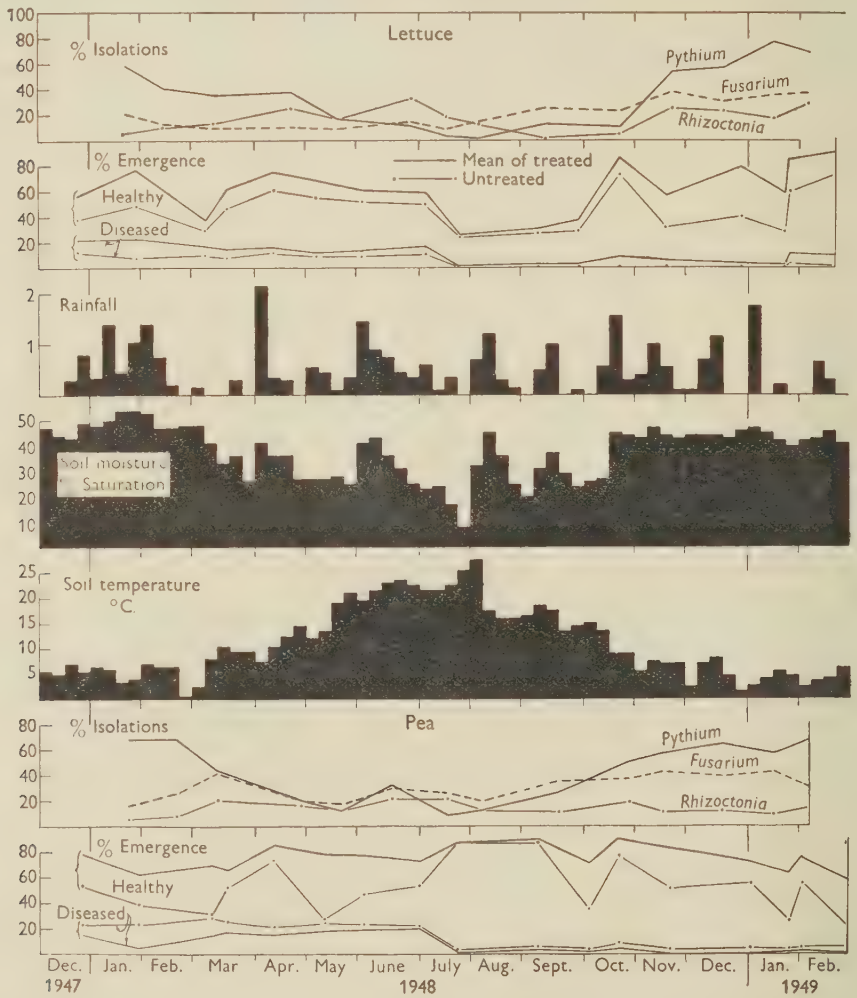


Fig. 3.

The seasonal variations in response for lettuce and pea are set out in Fig. 3, with data for temperature, soil moisture and rainfall.

For lettuce a favourable result is shown throughout the whole period but is at its minimum from about July to September when temperature was high and soil

moisture low. It is most pronounced during the winter months. The pronounced temporary drop in the curves at the beginning of March can be associated with wet and very cold soil conditions; the same applied in a less degree to the drops shown in mid-November and end of January.

TABLE 15. *Effect of temperature and soil moisture on emergence of lettuce in the field*

	Cool				Warm			
	Temperature 0–15° C.		W.H.C. 30–53 %		Temperature 15–30° C.		W.H.C. 10–30 %	
Treatment	H.	D.	H.	D.	H.	D.	H.	D.
Control	48·8	12·1	46·1	12·3	40·9	9·9	47·1	9·9
Cuprocide	56·8	9·3	56·7	9·1	43·0	7·2	43·1	7·6
Spergon	66·1	6·1	63·3	6·4	44·5	7·3	53·4	6·7
Panogen	56·4	8·0	54·8	7·9	41·1	8·2	44·1	8·5
T.M.T.D.	69·8	5·5	67·4	5·7	47·4	6·6	55·6	6·2
F.D.D.	59·0	7·7	57·0	7·8	42·3	8·0	46·4	7·8
Dow 9 B	54·7	7·6	52·8	7·6	41·5	7·0	45·3	7·0
36 L	65·6	5·6	65·6	5·6	—	—	—	—
Signif. at 5 %	3·2	0·9	3·4	1·0	6·2	0·8	5·1	0·7

TABLE 16. *Effect of temperature and soil moisture on emergence of pea in the field*

	Cool				Warm			
	Temperature 0–15° C.		W.H.C. 30–53 %		Temperature 15–30° C.		W.H.C. 10–30 %	
Treatment	H.	D.	H.	D.	H.	D.	H.	D.
Control	46·3	13·6	45·9	14·1	68·7	12·6	59·6	12·3
Cuprocide	57·1	12·1	50·8	9·9	74·7	12·9	71·0	10·1
Spergon	71·6	7·4	70·2	7·6	77·6	11·2	76·7	9·3
Panogen	66·4	11·7	59·7	9·5	73·5	10·3	70·9	8·7
T.M.T.D.	73·0	7·6	71·1	7·2	81·3	10·7	80·4	9·5
F.D.D.	65·5	12·1	58·1	9·9	76·5	12·0	74·5	9·7
Dow 9 B	58·2	11·3	50·6	9·1	75·5	11·2	74·1	9·3
36 L	71·8	8·1	57·0	9·0	—	—	73·9	9·7
Signif. at 5 %	3·8	1·5	4·4	1·8	4·8	1·6	3·9	1·4

The general trend of the curves is similar to that obtained by Sidky (1947) who worked with Cuprocide alone. His graph shows a much more extensive depressed region over the summer months, and it will be noted that his records relate to the hot and dry summer of 1947. Sidky also found that Cuprocide dressing gave a reduced stand as compared with the controls over the summer period, probably because conditions were drier in that year and he was working with a higher dosage (viz. 1 %).

The main feature of the graph for pea is the levelling up of emergence by the treatments in contrast to the up-and-down behaviour of the untreated lots. The

absence of a general depression over the period July to September, in contrast to that shown by lettuce, is probably to be set down to the fact that peas are sown much deeper in the soil than are lettuce so that the germinating seed are not so liable to the risk of desiccation.

The dates of emergence under cooler (0–15° C.) and warmer (15–30° C.) conditions were analysed separately and are given in Tables 15 and 16.

In general, field trials were not carried forward to allow for recording of yields, but some records of plant-growth, especially on pea, showed increase compared with controls. In some trials, however, carried out in co-operation with the National Pea Growers' Committee at Spalding and Cambridge, yield records were taken but did not give significant differences when compared with controls. These trials were sown in March 1948 and grew through a relatively dry spell which may explain that increased stand lacked moisture for a corresponding increase in yield. It may therefore be assumed that available moisture produced as many pods in the controls as in the treatments, whereas owing to increase in stand straw production was higher. These experiments were, however, carried out under conditions beneficial to emergence; under unfavourable conditions, stand of unprotected seed being drastically reduced, yield would decrease correspondingly.

The effect of mixtures of substances under test was tried but no advantage was obtained as compared with separate ingredients.

Effect of watering on stand of seedlings

Several authors, amongst whom L. K. Jones (1927, 1931) working with pea, Doran (1946) with tomato, lettuce, beet and cabbage, and Sidky (1947) with lettuce, found that application of water less than 24–28 hr. after sowing reduced stand of seedlings. Ledingham (1946), however, found no correlation between rainfall near the date of sowing and pre-emergence damage to pea seed. Lawrence (1948) reported *watering in* of seed to increase emergence of tomato and lettuce seedlings.

In field trials it was observed that a fall of rain soon after sowing, which increased soil moisture in the cold season (October–April) above the level of 40–50 % W.H.C. caused considerable reduction in emergence (Fig. 3).

In a series of greenhouse tests (16–20° C.) moisture was increased from an initial level of 25 to 45 % and from 45 to 65 % W.H.C. respectively. Measured amounts of water were added at regular intervals, i.e. immediately, ½, 1, 2, 4 and 8 days after sowing. One lot of treatments remained without addition of water. These experiments were carried out with all substances except 36L, and results are given in Tables 17 and 18.

In autoclaved soil, all systems of watering increased emergence at the low moisture level and all to much the same degree. Treated seed behaved largely like untreated. The low moisture content of 25 % was obviously the limiting factor to emergence. With autoclaved soils initially at 45 % W.H.C. addition of water, if delayed about

TABLE 17. *Effect of watering on emergence of lettuce (transformed values)*

Watering	Autoclaved soil								Unsterilized soil							
	25–45 %				45–65 %				25–45 %				45–65 %			
	Untreated		Treated		Untreated		Treated		Untreated		Treated		Untreated		Treated	
	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.
None	24·1	1·2	21·5	0·8	46·2	3·2	55·3	1·5	39·3	1·0	38·7	0·7	56·5	4·9	65·1	3·5
8 days	58·6	1·3	61·2	1·3	74·1	1·8	73·4	0·9	68·1	1·9	71·2	1·1	52·2	4·0	62·9	3·3
4 days	61·9	1·0	60·8	1·0	72·6	1·8	75·1	0·9	62·9	1·6	68·8	0·9	50·0	4·3	65·1	3·7
2 days	69·7	0·8	70·1	0·8	74·7	1·8	73·0	0·9	56·9	4·0	68·6	2·0	48·8	4·5	60·8	4·5
1 day	72·0	1·0	71·7	1·0	65·0	1·7	62·9	0·9	62·7	3·9	75·7	2·0	49·9	5·7	68·2	4·2
½ day	62·4	1·3	67·0	1·3	43·0	3·4	49·0	1·3	61·5	2·8	70·9	1·8	47·4	5·4	67·5	3·8
Immediately	64·9	1·0	64·9	1·0	42·2	2·3	48·5	1·5	56·8	1·9	63·5	1·2	48·1	5·3	56·7	3·9
Signif. at 5 %	5·7	0·9	5·7	0·9	8·2	0·8	8·2	0·8	10·2	0·9	10·2	0·9	5·8	1·3	5·8	1·3

—— = Signif. higher than controls. —— = Signif. lower than controls.

TABLE 18. *Effect of watering on emergence of pea (transformed values)*

Watering	Autoclaved soil								Unsterilized soil							
	25–45 %				45–65 %				25–45 %				45–65 %			
	Untreated		Treated		Untreated		Treated		Untreated		Treated		Untreated		Treated	
	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.
None	58·1	5·2	60·7	4·2	78·3	2·0	78·5	2·0	39·2	3·6	58·1	1·7	67·2	2·7	73·2	2·1
8 days	60·7	3·8	71·6	1·7	69·7	2·6	70·4	3·7	59·0	4·7	69·7	3·1	71·6	3·2	77·1	2·1
4 days	66·4	3·7	80·5	1·2	73·6	2·2	73·0	2·0	58·3	4·5	68·3	3·2	60·1	4·6	75·0	2·8
2 days	70·6	2·7	72·5	2·1	72·5	1·7	78·3	0·7	59·3	3·2	68·0	4·3	60·5	4·7	76·1	2·4
1 day	73·6	2·1	68·9	2·2	73·6	2·2	78·7	0·7	62·0	0·7	74·1	1·6	56·8	5·0	69·4	3·2
½ day	69·7	3·7	71·6	1·7	68·0	3·1	73·6	2·0	56·0	4·7	66·4	3·6	60·0	4·5	74·1	2·2
Immediately	63·4	4·6	68·9	2·5	68·9	2·3	73·6	0·7	55·6	3·3	70·6	0·7	45·0	5·3	79·2	3·0
Signif. at 5 %	3·7	1·5	3·7	1·5	6·7	1·3	6·7	1·3	11·0	3·5	11·0	3·5	7·8	2·3	7·8	2·3

—— = Signif. higher than controls. —— = Signif. lower than controls.

one day or longer, considerably increased emergence. Earlier additions had a depressing effect. Both treated and untreated seeds reacted similarly. In unsterilized soil additions of water over the range 25–45 % had the same effects as in autoclaved soil; at the higher moisture range, additions regularly depressed emergence for untreated seed. With treated seed there was a higher emergence throughout, with little variation resulting from the precise time at which water was added.

Table 18 gives the corresponding results for pea: in autoclaved soil the results are closely similar to those shown by lettuce. The levelling effect of treatments at the higher moisture level is, however, more pronounced. In unsterilized soil the results are substantially the same as for lettuce.

A similar experiment was carried out at the higher moisture level (45–65 % w.h.c.) with autoclaved soil inoculated with *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium solani* in equal proportions. The results are given in Table 19.

TABLE 19. *Effect of watering on emergence of lettuce and pea in inoculated soil*

	Lettuce				Pea			
	Untreated		Treated		Untreated		Treated	
Watering	H.	D.	H.	D.	H.	D.	H.	D.
None	78·0	10·0	90·0	2·0	5·0	15·0	80·0	10·0
8 days	68·0	0·0	82·0	2·0	5·0	15·0	65·0	0·0
4 days	38·0	0·0	72·0	0·0	0·0	15·0	50·0	0·0
2 days	24·0	12·0	74·0	0·0	5·0	0·0	40·0	0·0
1 day	28·0	6·0	64·0	0·0	0·0	5·0	25·0	0·0
½ day	20·0	2·0	58·0	0·0	0·0	10·0	35·0	0·0
Immediately	26·0	0·0	46·0	0·0	0·0	10·0	20·0	0·0

In this case the results are more striking, although they follow the same trend as in Tables 17 and 18. It will be noted that under these conditions untreated pea seed gave very low emergence whereas treatments were very beneficial. Even so, the effect of very high moisture (65 %) at the early stages could not be balanced by action of the protectants.

Two experiments along the same lines were carried out in the field at Slough. Cloches were used as protection against rain, and the same system of watering as outlined above was adopted, the application being at the rate of 2 gal./sq. yd. The effect was to increase the water content by about 20 %, but the increase in moisture tended to be temporary except where heavy rain fell within the period of tests and original soil moisture was high (viz. with bean). The data of emergence obtained in these two experiments were very similar and so they are combined in Table 20.

The general trend of the results is the same as that shown in Tables 17 and 18 for unsterilized soil at the higher moisture level. Treatments, as before, were uniformly beneficial (not all to a significant degree), and had a general levelling effect on the emergence shown under the various watering treatments.

Isolations were made from diseased seedlings in plots not watered and in those

TABLE 20. *Effect of watering on emergence of lettuce, pea, bean, turnip and beet under field conditions (transformed values)*

	Lettuce				Pea				French Bean				Turnip				Beet*			
	Untreated		Treated		Untreated		Treated		Untreated		Treated		Untreated		Treated		Untreated		Treated	
Watering	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.	H.	D.
None	45·7	8·3	56·6	7·0	55·3	13·5	61·1	12·0	57·0	17·5	62·7	12·6	53·0	1·6	65·7	1·2	267·2	4·6	333·5	3·4
8 days	42·0	7·2	51·9	5·3	61·0	11·1	65·1	10·5	66·1	10·5	76·9	10·5	58·1	1·1	61·4	1·3	319·4	1·1	315·4	2·9
4 days	43·2	9·9	47·8	6·0	52·9	22·6	64·6	13·0	64·4	8·4	76·4	8·6	52·1	1·5	59·2	1·1	249·5	2·5	310·9	3·0
2 days	37·5	9·1	50·3	7·6	55·1	10·4	71·9	11·0	53·3	16·5	71·1	15·1	45·0	1·6	52·0	1·1	225·0	1·2	330·5	1·4
1 day	36·9	8·7	55·9	7·7	54·5	10·1	70·6	11·1	56·7	23·2	64·3	13·2	43·0	1·2	49·5	1·0	219·5	1·8	303·5	2·1
½ day	32·8	7·3	49·5	7·0	54·8	11·7	69·8	10·4	44·2	25·7	66·4	16·8	42·0	1·1	50·4	1·1	155·2	4·9	325·7	2·5
Immediately	24·8	10·0	43·2	5·4	49·7	8·4	66·6	9·6	46·5	19·6	66·1	14·1	35·8	1·3	46·9	1·0	158·7	5·9	331·9	3·6
Signif. at 5 %	17·8	2·6	17·8	2·6	14·7	5·9	14·7	5·9	13·5	4·0	13·5	4·0	15·7	1·0	15·7	1·0	75·6	2·0	75·6	2·0

* From 300 seed balls.

—— = Signif. higher than controls. —— = Signif. lower than controls.

watered immediately after sowing. There was a considerable increase in percentage isolations of the main pathogens, especially *Fusarium* sp., on seedlings lifted from moist soil. It can be assumed that these pathogens are always present in the soil (McLaughlin, 1947), and that their growth is more rapid in moist soil (Virgin & Walker, 1939; Reinking, 1942; and others).

ISOLATIONS

A large number of isolations were made from seed which failed to germinate or which gave rise to inferior seedlings, and the genus of most of these identified by usual methods.

Table 21 illustrates the fungi isolated from seeds germinated on filter paper. The outstanding occurrence is the relative heavy infection of lettuce seed with *Rhizoctonia solani*.

TABLE 21. *Isolations from seed germinated on filter-paper*

Species	Lettuce	Pea	Bean	Turnip	Beet	Tomato	Celery
Fusarium	18·5	15·8	40·0	3·2	14·0	11·6	10·0
Rhizoctonia	13·5	1·5	1·0	0·0	1·0	0·0	2·0
Alternaria	1·8	5·4	3·7	6·4	0·0	14·2	1·0
Ascochyta	0·0	6·2	0·0	0·0	0·0	0·0	0·0
Botrytis	2·8	0·8	0·0	2·7	0·0	1·0	3·0
Phoma	0·0	0·0	0·0	11·0	38·0	9·9	6·0
Septoria	0·0	0·0	0·0	0·0	0·0	0·0	3·0
Bacteria	14·8	11·5	12·4	2·7	6·0	11·7	3·0
Colletotrichum	0·0	0·0	0·0	0·0	0·0	2·1	0·0
Cephalosporium	3·7	2·3	0·0	0·0	9·0	1·9	2·0
Stemphylium	3·7	2·3	0·0	0·0	8·0	1·0	0·0
Penicillium	2·8	6·2	22·8	2·7	8·0	8·5	1·0
Mucor	4·6	12·3	9·5	6·4	12·0	19·3	5·0
Pullularia	8·3	8·0	3·7	1·8	3·0	1·0	1·0
Acremoniella	0·0	0·0	0·0	0·0	0·0	0·0	4·0
Aspergillus	0·0	0·8	0·0	1·8	8·0	0·0	1·0
Rhizopus	2·0	0·8	0·0	0·0	0·0	2·0	0·0
Miscellaneous	0·9	2·3	3·7	0·5	2·0	1·6	0·0
No. of isolates	109	130	105	189	100	198	200

Probably contamination of the seed-heads largely arises from over-heaping and therefore slow drying of the cut seed-plants in the process of drying after harvesting (Hedgecock, 1904; Townsend, 1934; Elmer, 1942).

In a parallel series, isolations were made from similar seed germinating on malt agar (Ulster method). The same range of fungi was obtained but, with some at least (e.g. *Fusarium* sp.), in very different percentages from those shown in Table 21. Thus species of *Penicillium* are not usually present on a high percentage of seed as shown in Table 21, but the frequency on malt agar rose from 22 to 95 % on various seed lots. *Pullularia* sp., *Mucor* sp. and others behaved similarly. It would appear that the Ulster method gives undue encouragement to certain rapidly growing fungi which in themselves may have very little significance for germination of the seed.

Isolations were made from sowings at higher and lower temperatures 20 days after sowing.

The frequency of occurrence of *Pythium* was much reduced at the higher temperature but the opposite effect is shown by *Fusarium*. The effect on *Rhizoctonia* was slight and shows no definite trend.

A series of isolations was also made at the time of lifting from monthly sowings in the field. The frequency curves for *Pythium*, *Fusarium* and *Rhizoctonia* on lettuce and pea seedlings are given in Fig. 3, other crops showing similar tendencies.

The main trend shown, both for lettuce and pea, is the relative infrequency of *Pythium* during the summer period. It would be unsafe, however, to assume that these curves give a correct measure of the seasonal activity of *Pythium*, because it was found in subsidiary isolations that the percentage frequency of *Pythium* was much higher if the isolations were made at an early date after sowing. Thus the frequency of *Pythium* isolations at lifting time in the period June to October 1948 was from 2·5 to 13·0 %, but isolations 8 days after sowing showed percentages ranging from 38 to 85 %. The frequency of *Rhizoctonia* and *Fusarium* was less dependent upon the date chosen for making isolations. A similar tendency was observed in greenhouse tests when isolations were made 2–8 days or 20 days after sowing. It is apparent that there is a reduction in percentages of *Pythium* isolated at high temperature and low moisture conditions, but that this organism is never absent from the soil. There appears to be a distinct correlation between temperature and moisture and occurrence of *Pythium*, the frequency of this organism in isolations being determined by environmental factors (viz. low temperature and high moisture). It is therefore suggested that *Pythium* species tend to be obliterated by other organisms in the infected tissue and that this effect is more pronounced under summer conditions in the soil.

Seed disinfection

The efficiency of the seven seed protectants, at the standard dose used, was tested against a number of laboratory methods from the point of view of seed disinfection. The laboratory methods were:

1. Washing of seed for one hour under fast-running tap water.
2. Soaking for 10 min. in 0·1 % $HgCl_2$ dissolved in warm distilled water.
3. Soaking for 10 min. in 0·1 % acidulated $HgCl_2$.
4. Soaking for 10 min. in methylated ethyl alcohol.

After treatment seed was washed in eight to ten changes of sterile distilled water and dried between layers of sterile filter-paper.

Three hundred seed of each vegetable, treated by laboratory methods or by protectant substances, were then plated out on malt agar (Muskett & Malone, 1941). Table 22 gives the results in terms of percentage contaminated seeds for lettuce as typical.

TABLE 22. *Disinfection of lettuce seed*

	Contaminated seed (%)											
	Control	1	2	3	4	Cupro-cide	Sper-gon	Pano-gen	T.M.T.D.	F.D.D.	Dow 9 B	36 L
Botrytis	15·5	6	3	1	3	12·5	3	0	0	0	0	0
Fusarium	5	9	6	4	7	1	2·5	0	0	5	5	3
Rhizoctonia	7	5	2	0	1	9·5	0·5	0	0	0	0	0
Alternaria	5·5	10	8	2	5	8	1·5	0	0	0	0	0
Stemphylium	2	0	0	0	1	5·5	0	0	0	0	0	0
Pullularia	45	46	26	0	37	23·5	12	0	0	0	25	3
Penicillium	27	6	0	0	0	18	3	0	5	0	10	16
Cladosporium	27	4	3	0	0	18·5	2	0	1	0	10	1·5
Trichothecium	10	9	0	0	0	6	0	0	0	0	5	6·5
Mucor	4	12	4	0	0	17·5	1·5	1	0	0	5	5
Thamnidium	1	1	0	0	0	0	0	0	0	5	5	0
Cephalosporium	0·5	1	1	0	0	0	0	0	0	0	0	0
Rhizopus	8·5	4	0	0	0	7	0·5	1	0	0	0	0
Aspergillus	1	0	0	0	0	0·5	1·5	0	0	0	0	0
Others	28	4	0	0	0	23·5	4	0	0	0	0	0
Bacteria	18	25	18·5	4	15	10	1	0	17	20	21	19
Clean seed	0	0	57·5	71	11	0	85	92	63	70	45	38·5
Germination	83	93	94	46	34	95	98	95·5	99	90	90	95

The most effective agent for seed disinfection was Panogen, closely followed by Spergon; T.M.T.D. and F.D.D. were effective against many organisms but ineffective against bacteria. The three remaining proprietary substances were less effective.

Panogen was as effective in disinfecting lettuce seed as the best laboratory method (treatment with acidulated $HgCl_2$). Two of the laboratory methods, however, viz. 3 and 4, had a pronounced depressing effect on germination, a fault which none of the proprietary substances showed.

Similar records were taken of the other vegetables but these are not included. The results were in general similar to Table 22, apart from effects on particular organisms. None of the treatments was effective in preventing the growth of *Ascochyta* on pea, but they reduced *Fusarium* infection which may cause rotting of seed (Padwick, 1938). With French bean, no contamination by *Colletotrichum* was found in the controls; the most significant point is the effect of Spergon and Panogen in eliminating bacterial contamination which may cause seed decay (Winfield, 1949). With turnip, no treatment eliminated *Phoma*, but Panogen was again among the best. Infection with *Alternaria* was reduced by Spergon, Panogen and T.M.T.D., this organism according to Dennis (1939) being able to cause considerable damage to turnip seedlings. With beet, all the substances tested compared favourably with laboratory methods in reduction of the incidence of *Phoma betae* (Woodward & Dillon Weston, 1929; Heiberg & Ramsay, 1948). No specific organism was noted on the tomato seed, and though pycnidia of *Septoria* were present on 13 % of the celery seed used, the seed was over 2 years old and no growth of *Septoria* occurred in

any instance, seedlings included. This confirmed the recommendations of Leach & Houston (1943) on the effect of using celery seed more than 2 years old.

The adverse effect of seed treatment by the steeping process on germination capacity of seed has been confirmed throughout these tests, an effect reported earlier by Reinmuth (1934).

The treatments used in Table 22 were at selected dosages. Further experiments were carried out with dosages running up to maximum holding capacity. Some of these dosages were phytocidal (e.g. Panogen on all seed except lettuce and bean, Spergon on tomato and celery, T.M.T.D. and F.D.D. on tomato and Dow 9B on all seeds except lettuce decreasing germination), but the overall picture was the same as regards seed disinfection, viz. Spergon and Panogen were the most effective. None of these agents was able to eliminate or even appreciably reduce the growth of *Ascochyta pisi* from infected seed (as tested on seed showing pycnidial spotting which was selected from the bulk sample), germinated on nutrient agar. On the other hand all treatments (at 5 % of seed weight) except Cuprocide increased emergence of *Ascochyta*-spotted seed sown in autoclaved soil, as seen in Table 23.

TABLE 23. *Greenhouse test. Effect of treatments applied at* 0·5 % *by weight on emergence of* Ascochyta*-infected seed in autoclaved soil*

	Percentages from 100 seed	
Treatment	Emergence	Treated as percentage untreated
Control	53·4	100·0
Cuprocide	31·5	58·8
Spergon	69·4	129·5
Panogen	67·2	126·0
T.M.T.D.	57·8	108·2
F.D.D.	54·9	102·8
Dow 9 B	66·7	114·0
36 L	64·3	120·2

PATHOGENICITY TESTS

Preliminary tests were carried out and they will be dealt with in a summary form.

Linear growth of pathogens was measured on soil extract agar (pH 6·3) as prepared by the formula described by Lohnis (1913), to which were added nutrients suggested by Timonin (1940). Measurements of growth at temperatures ranging from 5 to 35° C. conformed, in general, with the known behaviour of these organisms, the most vigorous growth being shown by *Pythium*.

Effect of pH (as determined by the B.D.H. indicator method) at ranges from 5·5 to 8·6, showed inhibition of growth of *Pythium* on acid medium, that of *Rhizoctonia solani* and *Fusarium solani* not being affected.

Growth of *mixed colonies* showed a temporary slowing down together with thinning of hyphal strands where colonies met. After 24–48 hr., faster growing

organisms continued to spread over the slower growing colonies. These tests were carried out with *Rhizoctonia solani* and *Fusarium solani* inoculated simultaneously and *Pythium ultimum* inoculated after the others had a five days start (colony diam. 2·2–2·5 cm.) The same tests carried out on media adjusted to three levels of pH showed no difference in the general behaviour of colonies.

Substances such as Spergon, T.M.T.D., 36L, etc., *incorporated in the medium* (soil extract agar) at various concentrations, inhibited growth of *Pythium*, *Rhizoctonia* and *Fusarium* at dosages of 800–1000 p.p.m.

The relationship between growth rates of lettuce or pea and that of *Pythium*, *Rhizoctonia* and *Fusarium* was investigated. Leach (1947) in a temperature study brought forward a suggestion that these rates could be correlated with incidence of pre-emergence damping-off. The relationship is expressed by the coefficient of velocity of growth of these organisms on soil extract agar as percentage of the speed of emergence of lettuce or pea in autoclaved soil at the two levels of temperature used in the greenhouse and at soil moisture maintained at 45 % W.H.C. Results showed higher susceptibility of pea than of lettuce in soil inoculated with the three test organisms. For *Pythium* lower ratios were directly proportional with lower emergence, for *Rhizoctonia* and *Fusarium* the effect was reversed. In tests carried out at three soil moisture and two temperature levels the general conclusions reached were confirmed, and results suggest that the method of taking separate records for healthy and diseased seedlings is preferable to that of recording total emergence. As the main planting period falls within the ranges of temperature used in these tests, seed protection appears to be commendable as an insurance against soil-borne organisms of crops sown in all but the lowest levels of soil moisture.

For these tests soil was inoculated with each of the organisms separately or in a variety of mixtures. The method of procedure was as follows: A sterilized 5 % cornmeal sand mixture (190 g. sand + 10 g. cornmeal + 80 ml. water) was inoculated and this medium was permeated by fungal growth after 3 weeks' incubation at 25° C. Autoclaved soil was inoculated with 5, 10, 20 and 40 % by weight of the mixture thus prepared, care being taken to add sterile inoculum to controls. Mixing was done by hand followed by sieving. Inoculated soil was then held for 30 days at 16–20° C., after which it was again mixed to disperse mycelial growth on the soil surface. Mixed inoculum was prepared by adding soil inoculated with each separate organism in equal proportions and leaving the mixture for 10 days. This preparation was mixed again before use.

The effect of *progressively increased amount of inoculum* on emergence of pea is illustrated in Fig. 4.

The two fungi which have most prominently depressed emergence are *Pythium ultimum* and *Rhizoctonia solani*, and it is to be noted that the larger inoculum of the latter is less effective than the smaller. This type of result has been obtained by other workers (Sanford, 1941). The suggestion is that the large amount of organic material in the 40 % inoculum interferes with the pathogenic activity of the fungus. Difficulties

with *Pyronema confluens* (Pers.) Tul., have also been experienced, as this organism suppressed pathogenicity of inoculum. It may well be therefore that air-borne contaminants of a principally saprophytic nature are very difficult to exclude and that their action is encouraged by the organic material incidental to a heavy inoculum of the test fungus.

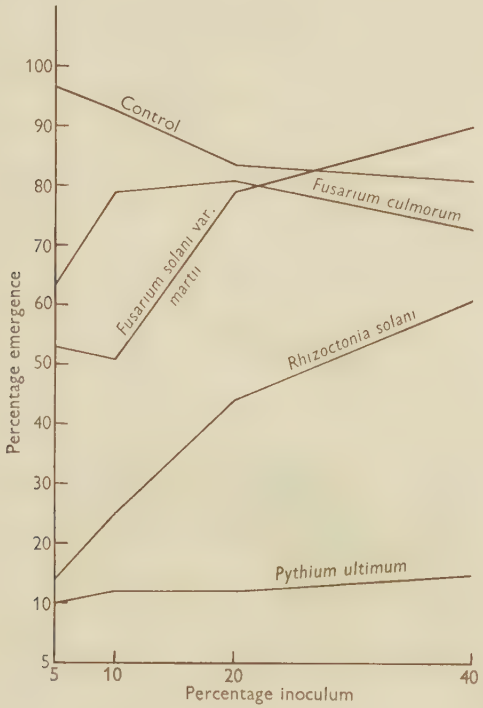


Fig. 4

Fusarium solani var. *Martii* behaved in a similar manner to *Rhizoctonia solani*, but was less pathogenic. The inoculum of *Pythium ultimum* was very active over the whole range tested. No depressing effect on emergence at any concentration of inoculum was observed with *Alternaria solani*, *Fusarium culmorum*, *F. oxysporum*, *F. sambucinum* and *Phoma lingam*.

Similar curves were constructed for the other vegetables. With great regularity *Rhizoctonia solani* gave greater emergence at the higher inoculum, the only exception being with French bean where the curve remained level throughout. French bean and turnip were less sensitive to *Pythium ultimum* than were other vegetables. This effect was noted by Winfield (1949) on French bean. Rather curiously, *Phoma lingam* inoculum was ineffective on turnip at the doses used. Another curious result was that no emergence of celery occurred in any of these inoculated soils.

For the testing of the various protective agents in artificially inoculated soil, the lowest dosage of inoculum, viz. 5 % was adopted as standard. *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium solani* var. *Martii* were used for further work, the latter having been reported by Ogilvie, Mulligan & Brian (1934) and F. R. Jones (1923) as being one of the organisms responsible for the rotting of pea seed.

Table 24 gives the effect of the selected seed protectants on emergence of lettuce and pea in soil inoculated with the three most important fungi.

TABLE 24. *Mean effect of protectants on emergence in inoculated soil*

	Lettuce							
	Un-inoculated		*P. ultimum*		*R. solani*		*F. solani*	
Treatment	H.	D.	H.	D.	H.	D.	H.	D.
Control	82·9	1·7	41·3	7·5	47·0	8·2	53·7	5·3
Cuprocide	80·5	1·0	54·7	4·0	59·5	0·9	60·2	1·0
Spergon	90·2	0·7	61·1	1·4	52·5	2·8	61·5	1·0
Panogen	80·3	2·7	48·8	3·0	52·3	1·3	60·5	1·7
T.M.T.D.	93·9	0·0	65·4	1·5	59·7	0·7	72·2	3·8
F.D.D.	84·1	0·6	55·0	1·1	51·0	0·8	58·2	1·3
Dow 9 B	85·3	1·1	48·5	1·3	48·8	5·8	64·8	1·5
36 L	85·5	1·9	69·9	2·3	60·0	0·3	69·5	2·7
	Pea							
	Un-inoculated		*P. ultimum*		*R. solani*		*F. solani*	
Treatment	H.	D.	H.	D.	H.	D.	H.	D.
Control	74·4	7·1	6·9	31·2	8·8	30·6	41·9	20·9
Cuprocide	76·3	2·3	50·9	6·3	49·1	10·0	70·8	2·7
Spergon	84·2	3·8	65·9	7·3	65·8	13·9	72·8	7·3
Panogen	79·8	2·7	43·2	6·9	38·1	14·7	64·3	5·1
T.M.T.D.	92·8	0·0	75·6	3·2	71·2	7·8	75·0	7·2
F.D.D.	85·5	1·8	48·6	3·3	46·3	10·3	65·5	5·6
Dow 9 B	84·8	0·6	48·1	2·9	51·1	4·5	64·7	5·5
36 L	85·8	7·5	69·3	8·7	74·3	9·7	60·3	6·0

Lettuce was less susceptible to the activity of pathogens than pea, and treatments were therefore more beneficial to the latter. T.M.T.D., 36 L and, to a less extent, Spergon appeared to be the most effective protectants. No special selective action of protectants on the soil fungi emerges from these tests.

The effect of temperature on activity of the three most active fungi is shown in Table 25. The figures are the means of all treatments (seed undusted and dusted with the various preparations).

This table confirms the conclusions that *Pythium* is more active at lower than at higher temperatures. *Rhizoctonia solani* and *Fusarium solani* show the reverse effect. Similar effects were observed by Greeves & Muskett (1936) on *Pythium*, F. R. Jones (1923) on *Fusarium*, and Richards (1923) and F. R. Jones & Lindford (1925) on *Rhizoctonia solani*.

TABLE 25. *Effect of temperature on emergence in inoculated soil (means from all treatments)*

	Lettuce				Pea			
	5–12° C.		16–20° C.		5–12° C.		16–20° C.	
Inoculum	H.	D.	H.	D.	H.	D.	H.	D.
Uninoculated	84·8	0·9	85·1	1·5	78·9	1·5	87·0	4·7
P. ultimum	48·3	3·9	60·1	1·7	40·7	8·4	61·4	8·8
R. solani	57·1	2·3	50·7	2·9	58·0	12·1	43·2	13·3
F. solani	62·7	1·5	59·9	3·1	67·0	5·5	61·8	9·6

The effect of moisture content in artificially inoculated soil was examined in the way already described for natural soil. The results conform with the conclusions already reached, viz. treatments had a levelling effect on emergence at the different moisture levels, whereas the emergence of untreated seed was much reduced by higher soil moisture.

The effect of using mixed inoculum is given in Table 26.

TABLE 26. *Effect of mixed inoculum on emergence of lettuce and pea*

	Lettuce		Pea	
Inoculum	Healthy	Diseased	Healthy	Diseased
Uninoculated	83·3	1·3	77·2	6·3
Pythium	44·0	7·3	8·5	28·5
R. solani	46·7	7·0	10·8	33·8
Pyth. + *Rhiz.*	55·7	5·3	14·7	28·7
F. solani	54·9	5·3	25·7	16·5
Pyth. + *Fus.*	42·7	7·0	21·2	24·5
Rhiz. + *Fus.*	66·7	4·7	19·8	20·7
Pyth. + *Rhiz.* + *Fus.*	60·0	5·0	16·5	19·5

These experiments have been repeated with like results, viz. that mixing of inocula does not increase the harmful effect upon emergence, and that in fact it often has the opposite effect, one organism apparently interfering with the activity of the other. The effect of temperature and soil moisture conformed with results previously described.

Mixing of protective substances gave no advantage over their separate use.

STORAGE TESTS

The effect of storing seed in open containers for 10 months was investigated, and comparisons were made of emergence of seed untreated and treated with selected protectants at standard dosages. These tests were carried out in unsterilized soil at 45 % w.h.c. and at 16–20° C.

Table 27 shows emergence as per cent untreated seed after storage.

TABLE 27. *Emergence of treated as percentage of emergence of untreated seed, after storage*

Treatment	Lettuce	Pea	French bean	Turnip	Beet	Tomato	Celery
Control	100·0	100·0	100·0	100·0	100·0	100·0	100·0
Cuprocide	109·5	121·8	127·0	61·5	105·0	89·5	97·7
Spergon	114·0	146·8	129·0	99·0	90·0	83·0	93·8
Panogen	91·5	133·0	131·8	79·5	104·0	100·7	95·0
T.M.T.D.	111·0	143·0	130·0	105·0	92·0	101·5	111·5
F.D.D.	109·0	136·0	127·0	89·0	86·0	103·0	103·0
Dow 9 B	104·0	132·0	122·0	80·0	92·0	94·5	111·5
36 L	110·0	153·0	124·0	92·0	98·5	119·0	122·0

With some exceptions (e.g. Cuprocide, Panogen and Dow 9B on turnip, Spergon on tomato) treatments as compared with untreated seed improved emergence of stored seed. This, however, was a general effect observed throughout this work. Similar effects of storage were observed by Baylis (1941) on wheat and Wilsie & Reddy (1946) on hemp.

Table 28 shows a comparison between stand after storage as percentage stand before storage.

TABLE 28. *Emergence of stored seed as percentage of emergence before storage*

Treatment	Lettuce	Pea	French bean	Turnip	Beet	Tomato	Celery
Control	99·5	95·0	87·5	89·0	90·8	91·0	87·0
Cuprocide	92·0	88·0	88·5	53·5	95·0	81·0	66·0
Spergon	98·0	108·0	99·0	82·0	98·5	70·5	80·0
Panogen	75·5	101·0	98·0	73·0	85·8	86·0	79·0
T.M.T.D.	99·0	100·5	98·8	94·5	90·0	103·7	94·5
F.D.D.	104·0	100·5	99·5	88·5	92·0	94·0	70·0
Dow 9 B	90·0	90·0	91·8	79·0	85·0	86·2	91·0
36 L	99·0	103·0	99·0	93·0	95·0	103·0	113·0

Moisture content of seed did not vary to any extent and results confirmed a statement made by Barton & Crocker (1948) that keeping quality of seeds stored at room temperature did not vary after 12 months provided the moisture content was lower than 15 %. With some exceptions (e.g. Cuprocide on turnip, Spergon on beet and Panogen on lettuce and turnip), stand was not considerably affected by storage for 10 months.

The general conclusion drawn is that seed treated within 12 months before sowing can be used without serious reduction in its commercial value.

DISCUSSION

The object in view was to examine the effect on vegetable seed of a considerable number of newly introduced seed protective substances, many of which are of a non-metallic nature and are devised to replace the older metallic (especially

mercurial) derivatives, to which there are objections on toxicological and other grounds. The testing followed lines usually adopted, viz. of preliminary assessment by laboratory and greenhouse methods, followed by extensive testing under varied soil conditions, both in the field and in soil which had been artificially contaminated with a variety of organisms.

Several laboratory methods have been tried, viz. slide germination, inoculation of plates with certain organisms and observation of effect of treated seed on checking mycelial growth, incorporation of substances into agar and inoculation of plates with certain organisms, etc. Although these methods gave some indication as to the efficacy of substances in controlling certain organisms, their applicability to the real practical problem is somewhat uncertain. Plating of treated seed (Ulster method) was found to be the most effective laboratory test, but even this method appears to be too drastic for several reasons: (1) that it gives a distorted picture of the microbial population on seed. Thus *Penicillium* spp., etc., are unduly encouraged so that by their vigorous growth they obscure slower growing organisms and thereby make difficult identification of the latter; (2) that it eliminates some substances, e.g. Cuprocide, which are effective protectants in soil, though ineffective on malt agar; (3) that it gives no practical indication of phytocidal effects of certain dosages, e.g. Panogen at 4 % of seed weight applied to lettuce gives a high germination on malt agar, whereas application of 2 % reduces emergence drastically when seed thus treated is sown in soil. It was therefore found that sowing on sand or filter-paper was the most effective method for assessing seed-borne organisms of any importance, whereas the selection of protective substances is most suitably carried out in soil tests.

Materials selected in such preliminary trials as would show their efficiency as disinfectants and protectants were then tested under an extensive range of conditions. It was seen in these tests that treatments brought about considerable increase in emergence under unfavourable weather conditions, but that stand was also increased even when emergence of the controls was generally high.

It can therefore be assumed that treatments which have been found effective in a prolonged series of experiments covering several seasons, in which observations were made on emergence disease reduction and freedom from toxic effects, should be applied to crops as a form of insurance against disease-producing organisms.

As has been stated above treatments considerably increased stand when germination was delayed and reduced by unfavourable weather conditions. It was also observed that treatments brought about greater resistance to post-emergence attack as was seen by the obvious reduction in numbers of diseased seedlings derived from treated seed. It can therefore be assumed that treated seed would bring about greater yield under such conditions.

Work here described was carried out only as far as recording the data of total emergence and the percentage of healthy and diseased seedlings in the early life of a crop. No record was therefore obtained of any subsequent infection or spread of

disease. Further evaluation may therefore show some degree of preference for one or more substances against others that have shown a similar degree of efficacy in emergence tests. Observations will therefore have to be extended to records of spread of infection, yield, etc., covering a wide range of environmental conditions. Evaluation of the optimum rate of application also appears to be part of the general scheme of selecting the most promising substances.

Some substances showed a certain amount of selectivity. Thus Cuprocide was toxic to turnip, Spergon to beet, etc. On the other hand Spergon was more beneficial to pea and Panogen to beet than when applied to others. It is possible that some substances may show selective effects in controlling specific organisms, whereas others may be found to be all-purpose fungicides. In this work T.M.T.D. was found to be the most effective substance generally.

Treated seed stored under dry conditions and for a relatively practical period, i.e. from one season to the next, showed no appreciable reduction of viability. Treated seed can therefore be used in the next season provided it is stored dry.

This work has proved that treatments can inhibit organisms in direct contact with the seed. How far such inhibition can extend through the soil from the immediate neighbourhood of the seed has not been assessed but it is reasonable to assume that there is some such action, as shown by the reduction in numbers of diseased seedlings.

The writer wishes to thank Professor W. Brown, F.R.S., for suggesting the problem and for helping in preparing the manuscript.

He is indebted to Dr A. J. Rutter for his assistance with the statistical analysis, to Mr H. Tooley for the taking of the photographs, and to all the manufacturing firms who supplied the materials listed in this work.

Appreciation is expressed to the authorities of the Imperial College of Science and Technology for the award of a half share in the Old Students' Royal College of Science Research Scholarship in Botany 1948–9.

This work has been made possible through a scholarship from the New Zealand Government Rehabilitation Department, to whom gratitude is expressed.

REFERENCES

ALBERT, E. D., HORSFALL, J. G., HEUBERGER, J. W. & STODDARD, E. M. (1941). Role of the dosage-response curve in the evaluation of fungicides. *Bull. Conn. agric. Expt. Sta.* no. 451, p. 635.

BARTON, L. V. & CROCKER, W. (1948). *Twenty years of seed research.* London, Faber & Faber, 152 pp.

BAYLIS, G. T. S. (1941). Fungi which cause pre-emergence injury to garden peas. *Ann. appl. Biol.* **28**, 210.

BAYLIS, G. T. S., DESHPANDE, R. S. & STOREY, I. F. (1943). Effect of seed treatment on emergence of peas. *Ann. appl. Biol.* **30**, 19.

BRETT, C. C. (1939). The production, handling, testing and diseases of seed. *Ann. appl. Biol.* **26**, 616.

Brown, W. & Montgomery, N. (1948). Problems in the cultivation of winter lettuce. *Ann. appl. Biol.* **35**, 161.

Clayton, C. N. (1948). Effect of several seed protectants on emergence and stand of okra. *Phytopathology*, **47**, 102.

Croxall, H. E. & Ogilvie, L. (1942). Experiments with protectant seed dressings, 1940–2. *Rep. agric. hort. Res. Sta., Bristol*, p. 65.

Dennis, R. W. G. (1939). Notes on seed transmission of *Phoma lingam* in relation to dry-rot of swedes in Scotland. *Ann. appl. Biol.* **26**, 627.

Doran W. L. (1946). Control of damping-off by a delay in first watering after seedling. *Phytopathology*, **36**, 679.

Doyer, L. C. (1938). *Leitfaden zur Untersuchung des Saatgutes auf seinen Gesundheitszustand.* Wageningen, H. Veenman and Zonen, 59 pp.

Elmer, O. H. (1942). Effect of environment on the prevalence of soil-borne Rhizoctonia. *Phytopathology*, **32**, 972.

Fisher, R. A. & Yates, F. (1948). *Statistical tables for biological, agricultural and medical research.* London, Oliver & Boyd.

Fitzgibbon, M. (1943). Seed disinfection. The determination of the adhesiveness of seed dressings to cereal seeds. *J. Soc. chem. Ind., Lond.*, **62**, 8.

Garrett, S. D. (1946). A study of violet root rot. *Trans. Brit. mycol. Soc.* **29**, 114.

Greeves, T. N. & Muskett, A. E. (1936). A temperature study of *Pythium* attack on swede seedlings. *Ann. appl. Biol.* **23**, 264.

Hedgecock, G. G. (1904). A note on *Rhizoctonia. Science*, **19**, 268.

Heiberg, B. C. & Ramsay, G. B. (1948). *Phoma* rot of garden beets. *Phytopathology*, **38**, 343.

Hulbert, D. & Whitney, P. M. (1934). Effect of seed injury upon the germination of *Pisum sativum. J. Amer. Soc. Agron.* **26**, 876.

Hull, R. (1937). Effect of environmental conditions, and more particularly of soil moisture upon the emergence of peas. *Ann. appl. Biol.* **24**, 681.

Jones, F. R. (1923). Stem and root-rot of peas in the United States, caused by species of *Fusarium. J. agric. Res.* **26**, 459.

Jones, F. R. & Lindford, M. B. (1925). Pea disease survey in Wisconsin. *Res. Bull. Wis. agric. Exp. Sta.* no. 64.

Jones, L. K. (1927). Studies in the nature and control of blight, leaf and pod spot and foot rot of peas caused by *Ascochyta* sp. *Bull. N.Y. St. agric. Exp. Sta.* no. 547.

Jones, L. K. (1931). Factors influencing the effectiveness of organic mercury dusts in pea seed treatments. *J. agric. Res.* **42**, 23.

Jones, L. R., Johnson, J. & Dickson, J. G. (1926). Wisconsin studies upon the relation of soil temperature to plant disease. *Bull. Wis. agric. Exp. Sta.* no. 71.

Keen, B. A. & Raczkowski, H. (1921). The relation between the clay content and certain physical properties of a soil. *J. agric. Sci.* **11**, 441.

Lawrence, W. J. C. (1948). *Science and the glasshouse.* London, Oliver & Boyd, 174 pp.

Leach, L. D. (1947). Growth rates of host and pathogen as factors determining the severity of pre-emergence damping-off. *J. agric. Res.* **75**, 161.

Leach, L. D. & Houston, B. R. (1943). Seed treatments for field and vegetable crops. *Circ. Calif. agric. Exp. Sta.* 5 pp.

Ledingham, R. J. (1946). The effect of seed treatment and dates of seeding on the emergence and yield of peas. *Sci. Agric.* **26**, 248.

Lohnis, F. (1913). *Laboratory methods in Agricultural Bacteriology.* Griffin & Co.

McCallan, S. E. A. (1947). The pea seed treatment method of evaluating fungicides in the greenhouse. *Phytopathology*, **37**, 15.

McLaughlin, J. H. (1947). The isolation of Pythium from soil at various seasons of the year as related to soil moisture and temperature. *Phytopathology*, **37**, 15.

McNew, G. L. (1943). Pea seed treatments as crop insurance. *Canner*, **96**, 13.

MUSKETT, A. E. & MALONE, J. P. (1941). The Ulster method for the examination of flax seed for the presence of seed-borne parasites. *Ann. appl. Biol.* **28**, 8.

MUSKETT, A. E. (1944). The short wet method of seed disinfection. *Ann. appl. Biol.* **31**, 218.

OGILVIE, L., MULLIGAN, B. O. & BRIAN, P. W. (1934). Progress report on vegetable diseases. VI. Diseases of peas. *Rep. agric. hort. Res. Sta., Bristol*, 110.

PADWICK, G. W. (1938). Complex fungal rotting of pea seeds. *Ann. appl. Biol.* **25**, 100.

REINKING, O. A. (1942). Distribution and relative importance of various fungi associated with pea-rot in commercial pea-growing areas in New York. *Tech. Bull. N.Y. agric. Exp. Sta.* no. 264.

REINMUTH, E. (1934). Beiträge zur Frage der Germüsesamenbeizung und zur laboratoriumsmässigen Prüfung der Beizungsmittelwirkung bei Gemüsesamen. *Angew. Bot.* **16**, 441.

RICHARDS, B. L. (1923). Soil temperature as a factor affecting the pathogenicity of *Corticium vagum* on the pea and bean. *J. agric. Res.* **25**, 431.

SANFORD, G. B. (1941). Studies on *Rhizoctonia solani* Kühn. V. Virulence in steam sterilized and natural soil. *Canad. J. Res.* **19**, 1.

SIDKY, S. T. (1947). Pre-emergence damping-off of lettuce seedlings: effect of environmental conditions on the persistence and activity of the parasite, with experiments on the value of copper fungicides as seed protectants. Ph.D. Thesis, London University.

TIMONIN, M. I. (1940). The interaction of higher plants and soil micro-organisms. The study of the microbial population of the rhizosphere in relation to resistance of plants to soil-borne disease. *Canad. J. Res.* **18**, 444.

TOWNSEND, G. R. (1934). Bottom rot of lettuce. *Mem. Cornell agric. Exp. Sta.* no. 158.

VIRGIN, W. J. & WALKER, J. C. (1939). Relation of temperature and moisture to near wilt of pea. *J. agric. Res.* **59**, 591.

WILSIE, C. P. & REDDY, C. S. (1946). Seed treatment experiments with hemp. *J. Amer. Soc. Agron.* **38**, 693.

WINFIELD, J. N. (1949). A study of certain disease problems arising in the cultivation of the French bean. Ph.D. Thesis, University of London.

WISHART, J. (1940). *Field trials. Their lay-out and statistical analysis.* Imp. Bur. Pl. Breeding & Genetics, 36 pp.

WOODWARD, R. C. & DILLON WESTON, W. A. R. (1929). Treatment of sugar beet 'seed' to prevent seedling diseases. *Ann. appl. Biol.* **16**, 542.

YOUDEN, W. J. (1940). Experimental designs to increase accuracy of greenhouse studies. *Contr. Boyce Thompson Inst.* **11**, 219.

(*Received* 3 *July* 1950)

MICROBIOLOGICAL CONTROL OF *BOTRYTIS CINEREA* PERS.

I. THE ROLE OF pH CHANGES AND BACTERIAL ANTAGONISM

By F. J. NEWHOOK

*Imperial College of Science and Technology, London, S.W.7**

(With 2 Text-figures)

Strains of *Bacillus*, *Pseudomonas* and *Chromobacterium* isolated from dead leaves of lettuce seedlings overwintering in the open have been proved to be antagonistic in various degrees to *Botrytis cinerea* on nutrient agar, on wounded detached lettuce leaves and on wounded leaves of lettuce seedlings at temperatures ranging from 4 to 25° C. under conditions of high moisture and humidity. A strain of *Micrococcus* was completely non-inhibitive. Antagonism by the strains of *Bacillus* and by *Bacterium coli* was evident only at temperatures above 15–20° C. while the other strains were effective also at temperatures as low as 4° C. Bacterial growth on agar and on dead lettuce tissue usually raised the pH from 6·1 to 7·8–8·4 at which level growth of *Botrytis cinerea* is almost negligible and the activity of any pectinase it produces is very much reduced. The antagonism of the bacteria investigated, however, was largely due to antibiotic substances quite independently of the effect of the high pH. Some bacteria which did not give rise to wide inhibition zones were nevertheless strongly antagonistic in the immediate neighbourhood of the colony. *B. cinerea* on nutrient agar and on lettuce leaves produced a strongly acid reaction. This was capable of neutralizing the alkaline reaction due to most bacterial activity only if the latter was not already well established. The mixed bacterial flora of all dead lettuce leaves tested was potentially inhibitory to *B. cinerea* to a level as high as or higher than that shown by the majority of the test strains in pure culture. Many strains of bacteria cause lysis of young active *Botrytis* mycelium and germ tubes. In association with many bacteria, *B. cinerea* has exhibited features such as stimulation or depression of sporulation, continuation of aerial growth with inhibition of lateral spread of mycelium, vesicular distortion of hyphae and germination of spores while still attached to the conidiophores. Some spores of the test strain of *B. cinerea* used gave rise to new strains more resistant to antagonism than the original.

Botrytis cinerea Pers. is probably the most important cause of mortality in winter lettuce in the British Isles. Germinating spores of the fungus do not readily attack healthy green leaves but if they germinate on dead tissue a rapidly spreading rot ensues, leading usually to death of the plant. The lettuce plant has no leaf-abscission mechanism and as about 8–10 outer leaves die normally before hearting occurs (apart from frost damage in severe seasons), all plants are potentially liable to infection through an accumulation of infectible tissue. As *B. cinerea* is a ubiquitous organism it is indeed surprising that there is any appreciable survival under these circumstances.

* Now Mycologist, Plant Diseases Division, Auckland, New Zealand.

Brown & Montgomery (1948) found that heavier soil gave consistently better survival of overwintering lettuce than lighter well-drained soils. Sowings in hollows consistently gave less *Botrytis* damage than sowings on ridges. They suggested that these conditions might induce better survival by virtue of increased microbiological antagonism, in particular against *Botrytis*, although the hollows would also offer greater protection of roots from frost damage.

The work described in this and a subsequent paper was undertaken primarily to determine the role, if any, of microbiological antagonism in the control of *B. cinerea* on lettuces overwintering in the field. A complementary aspect, viz. the possibility of using antagonistic organisms to control the disease was studied by Wood (1951) who has also reviewed the relevant literature.

Materials and methods

Throughout this work a strain of *Botrytis cinerea* isolated from a diseased lettuce seedling was used for all tests.

Lettuce extract was used in all *in vitro* antagonism tests. 250 g. of washed, rootless, healthy lettuce seedlings were boiled for 20 min. in 800 ml. of distilled water. The extract was obtained from the supernatant liquid and from squeezing the remainder in a muslin bag. It was clarified by passing through a pulp filter and made up to 1000 ml. Unless the liquid extract was required, 2 % agar was added. No other additions were made except to buffer when required. The pH was 6·1.

M/20 phosphate buffering was used. The appropriate amounts of solid potassium dihydrogen phosphate and sodium hydroxide calculated from the formula recommended by Clark (1920) were added to the media before autoclaving. Allowance was made for slight changes in pH during sterilization.

Routine determinations of the pH of solid media, plant tissue or liquids were normally made colorimetrically by crushing or mixing some of the material with an equal quantity of B.D.H. Indicator on a white tile. Occasional checks were made against a glass-electrode pH meter. Accuracy to 0·2 pH units was readily obtained by the visual method, particularly when a series of tests was being made. Periodical tests could be made on cultures without unduly exposing them to contamination.

During the course of the field trials it was often found necessary to test large numbers of seedlings for the presence of established *B. cinerea*. A rapid and simple method was to place seedlings whole in sterile Petri dishes with their roots under a segment of moist filter-paper. If active *Botrytis* was present, spores developed within 24–48 hr., whereas chance contamination did not lead to spore production before at least 5 days.

Other methods are more conveniently described in the body of the text.

THE RELATIONSHIP OF pH TO GROWTH AND ENZYMIC ACTIVITY OF *B. CINEREA*

Bacterial, fungal and actinomycete growth often caused a rise in pH of lettuce extract agar to 7·2–8·4, the alkaline zones often extending over 3 cm. from the colony. A few fungi had an acid reaction and some organisms did not materially alter the initial pH (6·1–6·2) of the medium.

It was therefore necessary to investigate the role of pH in the antagonisms often observed between *Botrytis cinerea* and other test organisms on lettuce extract agar.

Colonies of *B. cinerea* growing on lettuce extract agar produced an acid zone of pH 4·5 which extended 5–10 mm. ahead of and about 5 mm. behind the advancing edge of the colony. Behind this the pH rose to 5·8 at 10 mm. from the margin, thereafter rising rapidly to 6·7–6·9 at which value it remained steady.

The growth rates of *B. cinerea* on lettuce extract agar, unbuffered, and buffered at various pH values, at 18 and 6° C. are illustrated in Fig. 1.

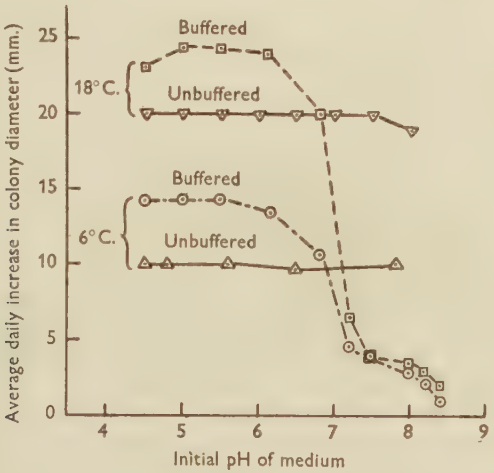


Fig. 1. The effect of pH and temperature on the growth of *Botrytis cinerea* on lettuce extract agar.

It will be seen that at either temperature the growth rate on medium initially buffered at pH 8·4 is only about 8 % of the rate at 4·5–6·1. Buffering at 8·4 was efficient only in maintaining a value of 8·0 against the acid drift due to *Botrytis* growth. Owing to this development of a strongly acid reaction, the growth rate of *B. cinerea* was not affected by the different original pH values of the unbuffered series of media.

Changes in pH comparable with those on the nutrient agar have been obtained by growing *B. cinerea* and other test organisms in pure culture on killed sterilized lettuce leaves.

Saprophytic activity on dead tissue of seedlings from the field kept for several days in humid Petri dishes always caused the development of a pH of 8·0–8·4 except in the initial stages of *Botrytis* attack.

Determinations of pH have been made from large numbers of samples of healthy, diseased and rotting lettuce leaves in the field. These are summarized as follows:

(1) Healthy green leaves	6·1–6·3
(2) Actively advancing *Botrytis* rot	4·3–6·0
(3) Sporulating, older *Botrytis* rot	5·6–6·9
(4) Dead tissue with no sign of *Botrytis*	6·0–8·4

In the last category, moist, well-rotted tissue almost invariably gave high pH values, e.g. 7·6–8·4.

Thus, despite the chemical and physiological changes consequent on the preparation of lettuce extract agar, the pH changes observed in connexion with the growth of *Botrytis* and other organisms on this medium are closely simulated on dead lettuce tissue under natural conditions.

Adjusting the pH from the optimum value 6·2 to 8·4 considerably reduced the activity of pectinase produced by spores of *B. cinerea* germinating in lettuce extract. This confirmed the work of Menon (1934), Fernando (1937), and Ashour (1949), who used various other media.

The role of bacterial antagonism to *B. cinerea* is discussed in the present paper, while the part played by fungi, actinomycetes and mixed saprophytes is the subject of a subsequent paper.

Bacterial antagonism to *Botrytis cinerea* under controlled conditions

After a preliminary test against *B. cinerea* ten apparently different strains of bacteria isolated from rotting lettuce leaves were selected for further testing. They represented all grades from highly antagonistic to non-antagonistic.

They will most conveniently be referred to in the text according to the following symbols:

*Bs*1, *Bs*2 and *Bs*3. Strains of the *Bacillus subtilis* group.

Ba. A strain of the *B. anthracis* type.

*Ch*1 and *Ch*2. Strains of *Chromobacterium* sp.

*Ps*1, *Ps*2 and *Ps*3. Strains of *Pseudomonas* sp.

M. *Micrococcus* sp.

1. *Antagonism tests on culture media*

(*a*) *Inoculation of plates with discrete colonies of* Botrytis cinerea *and bacteria.*

Several preliminary tests were carried out in which three bacterial streaks were inoculated near the periphery of a plate while *B. cinerea* was inoculated at the centre.

As the results were confirmed and extended by the experiment described below, no further reference to them will be made.

In the final experiment along these lines the lettuce extract agar medium (pH 6·1) was used in the following forms:

(1) Unbuffered, with Brom-Cresol-Purple added to a suitable visible concentration before autoclaving (mid-point brown at about 6·1, changing to yellow with acid and purple with alkali).

(2) Unbuffered ('U') with no indicator.

(3) Buffered ('B') also at pH 6·1 with no indicator.

Fig. 2 explains the method of inoculation of the plates.

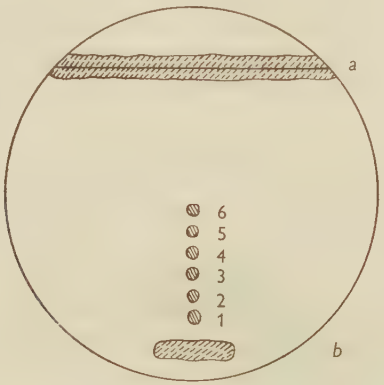


Fig. 2. Method of inoculation of plates: *a*, long bacterial streak + almost vertical cut; *b*, short bacterial streak; 1–6, *Botrytis cinerea* spore suspension droplets deposited with a six-point inoculating needle.

After 48 hr. of bacterial growth a cut was made with a sterile scalpel to cause the development of a bacterial barrier throughout the depth of the agar. As the cut was purposely made not quite vertical, low-power examination from below for penetration of the barrier by *Botrytis* mycelium was possible at all levels. It had been found in earlier tests that some strains prevented or reduced the passage of *B. cinerea* through the barrier while nevertheless allowing mycelium to pass below or over the surface of uncut streaks.

The bacteria were given 2 days prior incubation at 20° C. or 4 days at 4° C. After inoculating with *B. cinerea*, incubation was continued at the same temperatures. Control plates received *Botrytis* alone.

Germination records were made after 24 hr. and daily (20° C.) or on alternate days (4° C.) colony margins were traced in indian ink on to the backs of the Petri dishes, being retraced as a permanent record at the end of the experiment. Colour tracings of the plates incorporating Brom-Cresol-Purple were made on the 7th and

11th days, while colour changes were recorded more frequently in descriptive form: pH measurements were also made.

Buffering was effective to ±0·5 pH units against the strong alkaline and acid tendencies of the bacteria and *Botrytis* respectively. Some bacterial strains grew less well and *B. cinerea* grew somewhat better after the first day on buffered than on unbuffered agar.

With 48 hr. pre-growth, *Bs*2, *Bs*3 and *Ps*3 were the only strains which caused inhibition of germination at distances greater than 1 cm. at 20° C. during the first 24 hr. after inoculation of the *Botrytis* spores. *M* caused no inhibition even in drop 1 at 0·5 cm. *Bs*2 and *Bs*3 were also responsible for considerable vesicular distortion in drops 1–5. Inhibition was greatly reduced at 4° C. for all strains. *Bs*2 and *Bs*3 still induced slight vesiculation in drop 1 on unbuffered agar.

Allowing for slightly poorer growth of most of the bacterial strains on medium buffered at 6·1, it was evident that inhibition of *Botrytis* was due largely to factors other than the development of a high pH, even though the latter often reached an inhibitory level itself.

Strain *M* was responsible for a weak alkaline drift. With the remainder, the pH rose rapidly to 7·8–8·4. In the series with Brom-Cresol-Purple it was possible to observe the changes in reaction in the antagonism zones. In the case of the short streaks, acid production by the *Botrytis* advancing from the nearby inoculation drops neutralized the alkaline reaction of strains *Bs*1, *Ps*1, *Ch*1, *Ch*2, and *M*. However, the alkaline zone about the long streaks of all except *M* was maintained against the approaching *Botrytis*, even in the case of *Bs*1, *Ps*1, *Ch*1 and *Ch*2 where the *Botrytis* continued to advance, though at a reduced rate, up to the bacterial streak. Nevertheless, as seen from Table 1 and from a comparison of daily growth rates on buffered and unbuffered media, the main inhibitory effect was due to the production of antibiotic substances and not to the high pH produced by the bacteria.

Table 1. *Width of inhibition zones measured in mm. from the almost vertical cut—after 14 days at 20° C. and 24 days at 4° C.*

U = unbuffered; B = buffered agar.

Strain		20° C.	4° C.
*Bs*3	U	23	0
	B	23	0
*Bs*2	U	20	0
	B	20	0
*Ps*3	U	3	1–2
	B	3	1–2
*Ps*2	U	1–2	0
	B	1	0
*Ch*2	U	1	0
	B	0	0

Sporulation of *Botrytis* was stimulated by proximity to all strains except *M* and in some cases this tended to be greater on unbuffered than on buffered media.

Although strains *Ch* 1 and *Ch* 2, on buffered and unbuffered agar, both at 20 and 4° C., prevented mycelial penetration of the bacterial colony, *B. cinerea* nevertheless grew over the top as abnormal aerial mycelium.

Penetration of the more or less vertical bacterial barrier by *B. cinerea* is recorded in Table 2.

TABLE 2. *Relative frequency of penetration by* Botrytis hyphae *of the vertical barrier of bacteria—after* 14 *days at* 20° C. *and* 24 *days at* 4° C.

Strain no.		20° C.	4° C.
Control	U	+++	+++
M	B	+++	+++
Bs 1			
Ps 1	U	o	o
Ch 1	B	+	+
Bs 2	U	o	++
Bs 3	B	o	++
Ch 2	U	o	+
Ps 2	B	o	++
Ba	U	o	o
	B	o	+
Ps 3	U	o	o
	B	o	o

By the usual standards only *Bs* 2 and *Bs* 3 (and *Bs* 1 at temperatures greater than 20° C.), producing inhibition zones of 2 cm. or more (see Table 1), would be regarded as important fungistatic strains. However, under natural conditions action at a distance is probably less important than the mutual effects of organisms more or less in actual contact. By means of the above technique, as shown in Table 2, strains *Ps* 1, *Ch* 1 and *Ch* 2, which produce no inhibition zones on the surface, are nevertheless almost completely effective at 20 and 4° C. in preventing *Botrytis* from penetrating the actual colony. Similarly *Ps* 3, tested by the barrier technique, is the most effective inhibitory strain at both temperatures, yet its inhibitory effect is exerted only in a very narrow zone. Presumably these bacteria produce antibiotic substances that are non- or slowly diffusible, readily labile but continually replaced, or rapidly neutralized by substances in the medium so that they are effective only in the immediate vicinity of the colony.

(*b*) *Inoculation of* B. cinerea *on to bacterial colonies*

In view of the above, and as bacteria and *Botrytis* are in mutual contact on dead tissue, the following experiment was set up:

Plates of buffered and unbuffered lettuce extract agar were divided into two segments by the removal of a central strip. The first segment in each plate was

inoculated with a 2 cm.-square colony of bacteria from a 2-day old slope on the same medium. After 2 days' incubation a similar square was placed on to the second segment. A drop of *B. cinerea* spore suspension was then inoculated centrally on each square. Inoculations on the first set of segments will be referred to as the '2-day' and in the second set as the 'simultaneous' series, i.e. with regard to the age of the bacterial colonies at the time of inoculation of *B. cinerea*. Parallel series were set up at 15°, 20° and 25° C.

An adaptation of the same technique was used in a series run at 4° C. so that three segments per plate were inoculated at 4-day intervals to provide colonies which will be referred to as '8-day', '4-day' and 'Simultaneous', relative to inoculation of *B. cinerea* and irrespective of their subsequent increase in age.

Examinations were made daily for 4 days and then on the 8th and 14th days (15–25° C.) or the 7th, 9th, 11th, 15th and 24th days (4° C.). Records included: (1) growth and motility of bacteria; (2) pH changes; (3) germination rate and subsequent growth of *Botrytis*; (4) bacterial lysis of surface mycelium; (5) sporulation of *Botrytis* on the bacterial colonies.

A strain of *Bacterium coli* was included for comparison with the other test strains.

The pH value for buffering was chosen as 6·9 to obtain satisfactory bacterial growth. It was considered more important to leave the unbuffered medium at the same pH (6·1) as that of newly dead tissue than to correct to 6·9. Direct comparisons between the two media in any case were liable to error due to the effects of the inclusion of the buffering materials, while the activity of the test organisms rapidly effected a much more significant change in the pH of the unbuffered medium.

Using pH changes as a measure of successful growth by the bacteria, Table 3 illustrates the effect of temperature on the test strains.

TABLE 3. *Effect of temperature on growth of bacteria as measured by rise in* pH

	No. of days for pH to reach 7·8–8·4			
Strain	4° C.	15° C.	20° C.	25° C.
B. coli	—	6	5	2
Bs 1	—	6	5	2
Bs 2	—	4–5	3	1–2
Bs 3	—	4–5	3	1
Ba	—	—	4	2
Ps 1, *Ps* 2, *Ps* 3	2	2	2	1–2
Ch 1, *Ch* 2	2	2	2	2

It will be noticed that apart from their lack of growth at 4° C., the first five strains in Table 3 all exhibited a maximum growth rate at 25° C., whereas the last five

strains showed no appreciable increased growth rate with rise of temperature. *Ch* 1 and 2 and *Ps* 1, 2 and 3 will be referred to hereafter as the 'low-temperature group' of bacterial strains. Cell motility was still vigorous at 4° C. in this group during the period of active growth. *M* grew moderately well at 15° C. and well at 20–25° C., but did not cause an extensive pH change. As it proved to be non-inhibitive under all conditions little further reference to it will be made.

In almost every case initial bacterial growth was apparently much more rapid in the cases of simultaneous inoculation, probably due to extra nutrient in the spore suspension (to avoid chance contamination spores were not washed), to synergistic effects between bacteria and *Botrytis* or to the additional moisture from the spore drop. In all cases where bacterial growth was vigorous *Botrytis* failed to reduce the high pH.

TABLE 4. *Inhibition of germinating* Botrytis *by established colonies of bacteria on unbuffered (U) and buffered (B) lettuce extract agar. Measurements taken* 40 *hr.* (15–25° C.) *and* 84 *hr.* (4°C.) *after inoculation of* Botrytis *spore suspension*

	Percentage reduction of maximum germ-tube length									
	4° C.				15° C.		20° C.		25° C.	
	8-day		4-day		2-day		2-day		2-day	
Strain	U	B	U	B	U	B	U	B	U	B
Bs 2	—	—	—	—	80	92	98	100	100	100
Bs 3	—	—	—	—	90	85	96	88	99	98
Bs 1	—	—	—	—	75	0	0	0	94	94
B. coli	—	—	—	—	0	0	46	0	85	91
Ba	—	—	—	—	72	39	62	70	96	96
Ps 3	93	94	98	99	90	95	88	85	97	97
Ps 1	87	90	90	85	91	85	85	70	97	91
Ps 2	80	88	85	88	92	62	85	70	96	96
Ch 1	50	70	75	70	72	62	54	50	91	85
Ch 2	70	75	85	70	75	62	54	30	91	91

Table 4 illustrates the effect of temperature and buffering on the germinating *Botrytis* inoculated on to well-established bacterial colonies. According to their ability to inhibit *Botrytis* at 4° C. strains may still be segregated into the same two groups. Those in the first group could be expected to be of little importance in the field under winter conditions. *Bs* 1 and *B. coli* in particular were relatively ineffective at temperatures below 25° C. In the low-temperature group, the percentage inhibition at 4° C. was in all cases of the same order as that at higher temperatures, with little difference between 4-day and 8-day colonies of the same strain. *Ps* 3 was highly antagonistic at all temperatures from the 1st day; other strains normally did not give their maximum inhibition until the second day after inoculation of *B. cinerea*.

Table 5 presents the data for inhibition of *Botrytis* germination in the series of simultaneous inoculations. It will be seen that on both unbuffered and buffered media inhibition by *Bs* 1, 2 and 3 and *Ba* increased with rise in temperature. This was parallel to the increase in growth rate over the same temperature range, as shown in Table 3. *B. coli* was an exception, showing if anything a decrease in effectiveness with rising temperature and increased growth rate. In the low temperature group there was only a relatively slight increase in inhibition between the extremes at 4 and 25° C. This again is understandable from a comparison with Table 3. However, there was a sharp decrease in inhibition at 20° C., the optimum temperature for *B. cinerea*.

Except at 20° C., the percentage inhibition shown by the low-temperature group on the 2nd day of recording in the simultaneous series was essentially of the same order as that exhibited on the 2nd day of recording in the 2-day series, i.e. attainment of the maximum inhibitive potential, relative to the age of the bacterial colonies, was more rapid in the case of simultaneous inoculation of bacteria and *B. cinerea*. This was parallel to the more rapid growth in the simultaneous series as shown by the observations on pH described above, though independent of pH changes as it was equally striking on buffered agar.

After the first 48 hr., in cases where there was incomplete inhibition, germ tubes showed a tropic response away from the bacterial film, either penetrating the agar or developing as aerial mycelium. The latter usually grew more rapidly than the surface or submerged mycelium.

In a few cases where the majority of germ tubes failed to emerge from the bacterial squares, a few apparently more resistant germ tubes did emerge. Of some subcultures of these, when tested against the corresponding bacterial strain, four proved to be approximately 50 % more resistant to inhibition than the stock strain of *B. cinerea*. Others were indistinguishable in reaction.

In both series of the above experiment there was no definite indication that buffering to avoid a high pH materially reduced the degree of inhibition by any bacterial strain.

Sporulation of *B. cinerea* within the limits of the bacterial colony was invariably reduced, except with strain *M*, at all temperatures at which the test bacteria grew satisfactorily. Except in cases of complete suppression of sporulation and with all strains except *M*, *Bs* 1 and *Bacterium coli*, spores regularly germinated on the conidiophores. This also occurred occasionally with *Bs* 1 and *B. coli*.

Apart from considerations of the inhibition of growth of *Botrytis cinerea* the most important feature of this experiment was that lysis of young, active *Botrytis* mycelium resulted from contact with all except two strains (*M* and *Ba*). Bacterial cells were observed to concentrate in layers about the spores, germ-tubes and mycelium, possibly indicating more rapid division in their vicinity. This was followed by vacuolation of the *Botrytis* and finally by disappearance of the protoplasm. In many cases there was also disintegration of the cell walls.

TABLE 5. *Inhibition of germinating* Botrytis *after simultaneous inoculation with bacteria. Measurements taken* 40 *hr.* (15–25° C.) *and* 84 *hr.* (4° C.) *after inoculation of* B. cinerea *spore suspension*

	Percentage reduction of maximum germ-tube length							
	4° C.		15° C.		20° C.		25° C.	
Strain	U	B	U	B	U	B	U	B
Bs 2	—	—	10	33	42	25	80	84
Bs 3	—	—	30	13	42	25	90	84
Bs 1	—	—	0	0	0	0	73	73
B. coli	—	—	10	33	23	25	0	12
Ba	—	—	30	0	23	20	93	89
Ps 3	80	85	94	92	82	70	97	97
Ps 1	80	70	85	60	54	50	88	91
Ps 2	60	65	85	67	54	20	93	91
Ch 1	65	60	90	67	42	20	93	89
Ch 2	70	70	45	47	23	35	83	84

Table 6 summarizes the effect of temperature on the time required for lysis to commence.

TABLE 6. *No. of days after inoculation of* Botrytis *required for lysis to commence*

Strain	4° C.	15° C.	20°C.	25° C.
B. coli	—	2	2	2
Bs 1	—	—	—	1
Bs 2	—	2	1	1
Bs 3	—	2	1	1
Ps 1	3	1	2	2
Ps 2	4	1	2	2
Ps 3	3	2	2*	1
Ch 1	3	1	1	1
Ch 2	4	2	2	1

* Bacterial attack starting on ungerminated spores—no germination had occurred by this time.

In contrast to the lack of any apparent effect on growth and inhibition in the low temperature group, a temperature of 4° C. did delay the onset of lysis.

Although no quantitative measure of lysis was possible with this technique, lysis in most cases appeared to be more active on buffered media. On both media it often caused aerial mycelium to collapse to the surface enabling further lysis to take place.

(*c*) *Cylinder plate tests*

Culture extracts from the above bacterial antagonists were tested by an orthodox cylinder plate technique but the method did not give as much information about the antagonistic potentialities of all the test strains as those described above.

(*d*) *Pour-plate tests*

A modified pour-plate technique was devised to test on a large scale the possibility of there being a correlation between the absence of *Botrytis* from some dead leaves and the presence of a more antagonistic bacterial flora on those than on infected leaves.

A small piece of tissue was well crushed in 1 ml. of sterile distilled water and pour-plates made at different dilutions, using buffered and unbuffered lettuce extract agar. When these had set, a sterile sealing layer was poured on to prevent spread of surface bacteria in the moisture of the *Botrytis* spore suspension which was sprayed on after incubation of the plates for 48 hr. at 20° C. After a further 24 hr. at the same temperature, measurements of pH and germ-tubes were recorded. A final examination was made on the 5th day.

The pure culture test strains were submitted to this technique to provide a standard for comparisons. Data were recorded for seeding densities of 1–5, 5–10, 10–20, 20–50, 50–100 and > 100 colonies per low-power microscope field. Twenty-four hours after spore inoculation there were marked differences in the percentage inhibition of *Botrytis* by different strains at different densities. *M* was non-inhibitive throughout; the others gave complete control at seeding densities of > 100. With strain *Bs* 2 complete inhibition of *Botrytis* was shown even at a seeding density of 1–5. However, with further incubation all *Botrytis* growth ceased with all strains except *M*.

The technique was used in three separate experiments for the examination of the saprophytic flora of seventy-two dead lettuce leaves, thirty-five infected with *Botrytis* and thirty-seven free from infection as shown by the humidity dish test.

The results are summarized as follows:

(1) The mixed saprophytic flora of all leaves tested was potentially inhibitory to *B. cinerea* to a level as high as that shown by the most antagonistic test strains in pure culture.

(2) There was thus no correlation between the antibiotic potentiality of the saprophytic flora and the presence or absence of *Botrytis* on the leaf.

(3) Fungi developed in many plates, but their frequency and rate of development were such that their effects could not have been very significant compared with those of the bacteria.

(4) From results on buffered media it was obvious that the major inhibitory effect was due to antibiotic substances independently of high pH.

(5) *B. cinerea* present as spores or on debris in the suspension was able to grow and sporulate although it was almost always restricted in extent to its centre of origin, colonies often being represented by a single weak conidiophore.

Limitations of the technique:

(1) The nutritional and spatial relationships, etc., of the bacteria are obviously different from those on the leaf.

(2) It is impossible to standardize accurately the seeding rate of the pour-plates, even after partial filtering, owing to leaf materials in suspension, variable cell size and opacity of the bacteria, etc. This was overcome by setting up a wide range of seeding densities in the controls (i.e. pure cultures) against which to compare three standard dilutions from leaf material.

(3) Mutual antagonism between colonies in mixed cultures possibly causes antagonistic strains to predominate with pour-plates and on the leaf during the period in humidity. However, this is equally likely to happen in the field.

2. *Antagonism tests on lettuce leaves and plants*

After preliminary tests in conjunction with Dr R. K. S. Wood the technique described by him (Wood, 1951) was used. This involves the surface sterilization of lettuce leaves killing small areas with solid carbon dioxide and inoculating these lesions with *Botrytis* and antagonists.

Each of nineteen lesions per test strain was inoculated with bacteria, control lesions receiving sterile distilled water. After 3 days on the laboratory bench (25–30° C.) each lesion was inoculated with *B. cinerea*. After a further three days a few lesions had begun to increase in size (referred to hereafter as 'progressive lesions') and from the 4th day onwards records of lesion size were taken.

At the conclusion of the experiment the pH of the central lesion was measured. *Botrytis* spored freely on all progressive lesions.

Results are presented in Table 7. The final column indicates the relative efficiency of the test strains in preventing attack by *B. cinerea*.

There was a tendency in these tests for a few slightly older leaves to undergo a physiological collapse before completion of the test. This condition, often originating near the killed zone, was easily distinguishable from *Botrytis* rot by its lack of regularity, the coherence of the tissue, different colour and lack of sporulation.

In an earlier experiment, at 4° C., bacteria were inoculated 4 and 8 days before *B. cinerea* on lesions on the upper leaf surfaces. There were twelve replications in each series. There was no inhibition by bacteria in the 4-day series and while there was little complete control of *B. cinerea* in the 8-day series some strains caused a considerable increase in the time taken for rotting.

In another test the bacteria were inoculated (lower surface) one day before *B. cinerea* and incubated at 20° C. There were nine replications (see Table 8).

In a further test, with twenty-five replications, bacteria were inoculated two days before *Botrytis* and plates were kept on the laboratory bench (20–25° C.) (see Table 8). In the latter test, two control lesions failed to rot. An examination showed them to be heavily contaminated with bacteria.

The same technique was applied to a test on leaves still attached to plants in pots under bell jars standing in water, at a temperature of 20–25° C. Vaseline rings and inoculation drops were necessarily on the upper surfaces of leaves. There were

TABLE 7. *Inhibition by bacteria of rot due to* B. cinerea *on lettuce leaves after prior incubation of bacteria for* 3 *days* at 25–30° C.

Strain	Mean pH on 7th day	Extent of *Botrytis* rot: 4th day L.	M.	S.	N.	5th day L.	M.	S.	N.	6th day L.	M.	S.	N.	7th day L.	M.	S.	N.	% total inhib.*
Control	6·4	13	4	2	—	19	—	—	—	19	—	—	—	19	—	—	—	0
Bs 1	6·4	14	1	3	1	17	—	1	1	19	—	—	—	19	—	—	—	0
BS 2	8·0	—	1	1	17	1	1	1	16	3	4	—	12	7	1	—	11	58
BS 3	8·0	—	1	1	16	1	3	1	14	5	—	1	13	5	1	1	12	63
Ba	7·8	1	2	3	13	7	3	2	7	9	4	—	6	13	1	—	5	26
Ps 1	7·8	1	5	3	10	7	2	—	10	9	2	—	8	11	—	—	8	42
Ps 2	8·2	4	2	1	12	6	1	1	11	11	—	—	8	11	1	—	7	37
Ps 3	8·2	2	1	3	13	4	4	—	11	9	1	—	9	10	1	—	8	42
Ch 1	7·8	—	4	5	10	10	4	1	4	15	1	—	3	16	—	—	3	16
Ch 2	7·4	1	2	5	12	10	4	—	5	15	1	—	3	16	1	—	2	11
M	6·1	13	3	3	—	17	1	1	—	19	—	—	—	19	—	—	—	0

L. = lesions > 2 cm. diam.; M. = 1·5 – 2 cm.; S. = < 1·5 cm.; N. = no spread from the CO_2 lesion.

* Calculated from the number of lesions at the end of the experiment showing no progressive rot.

TABLE 8. *Summary of results of tests for inhibition of* Botrytis *attack by bacteria after prior incubation for different periods and at different temperatures*

Strain	Percentage total inhibition on detached leaves: 4° C. (8 days*)	20° C. (1 day)	20–25° C. (2 days)	25–30° C. (3 days)	Percentage total inhibition on living plants 20–25° C. (3 days)
Control	0	0	8	0	9
Bs 1	8	0	60	0	0
Bs 2	0	0	59	58	36
Bs 3	0	0	59	63	45
Ba	17	75	64	26	0
Ps 1	0	60	48	42	23
Ps 2	8	20	40	37	56
Ps 3	0	45	56	42	34
Ch 1	0	60	46	16	30
Ch 2	17	45	34	11	0
M	0	0	19	0	0

* Interval between inoculation of bacteria and *B. cinerea*.

eleven replications and bacteria were inoculated three days before *Botrytis*. As considerations of space and the advantage of utilizing leaves of different ages demanded that each plant should have more than one lesion, infected leaves were removed as soon as lesions entered the petiole. Records were taken daily until there was no further increase in the development of rot.

The final figures for all the above experiments are incorporated in Table 8.

Discussion

Under natural conditions soil organisms probably play the major part in the colonization of old or frost-damaged lettuce leaves, the normal points of infection by *Botrytis cinerea*. In a subsequent paper evidence will be presented to show that the antibiotic activity of mixtures of organisms of the soil flora against *B. cinerea* on this dead tissue is largely responsible for the survival of lettuce overwintering in the field. The work described in the foregoing pages is part of the preliminary investigation of the activity of individual organisms of the saprophyte complex.

In view of the low temperatures prevailing in the open during the winter it is interesting to note that strains of *Pseudomonas* and *Chromobacterium* were still actively antagonistic to *Botrytis cinerea*, at least *in vitro*, at 4° C. At this temperature there was also some slight evidence of control by the *Bacillus* test strains. This was unexpected in view of the observed and usually recognized temperature limits for growth within this genus. The explanation may lie in the possibility of a few cell divisions occurring before chilling was complete or in the transference of a sufficient antibiotic substance in the inoculum to account for the slight inhibition recorded. Such sources of error could be overcome by refinements of technique. However, it is not considered that errors from this source were very significant compared with the major effects shown by the low temperature group.

Despite the promise shown by the 'low-temperature' group of bacteria *in vitro* at 4° C., these organisms gave very poor control at that temperature on lettuce leaves although they were effective at 25–30° C. The differences between the *in vitro* and the leaf tests may be accounted for partly by the following considerations:

(1) Cuticle and cell walls probably impede rapid bacterial colonization of dead tissue and subsequent diffusion of antibiotic substances as compared with agar cultures. Different strains of bacteria probably differ in this respect, depending on individual enzyme complexes, motility, etc., and it is probable that some mixed cultures might be more successful in colonization through a division of labour.

(2) The level of local available nutrient and its nature, as compared with lettuce extract agar, are often possible limiting factors in the establishment of antagonism on leaves.

Other aspects such as the significance of the high pH produced by saprophytic activity *in vitro* and *in vivo* to control of *Botrytis* and the relationship of moisture to control *in vivo* will be discussed in the next paper after the presentation of further data.

The writer wishes to express his gratitude to Professor W. Brown, F.R.S., for suggesting the subject for this research and for providing much helpful criticism during its course; also to Dr S. E. Jacobs for advice and criticism in connexion with the section on bacterial antagonism.

The work was carried out during the tenure of a New Zealand Government Rehabilitation Bursary.

REFERENCES

Ashour, W. E. (1940). Ph.D. Thesis London.

Brown, W. & Montgomery, N. (1948). Problems in the cultivation of winter lettuce. *Ann. appl. Biol.* **35**, 161.

Clark, W. M. (1920). *The determination of hydrogen ions.* Baltimore: Williams and Wilkins.

Fernando, M. (1937). Studies in the physiology of parasitism. XV. Effect of the nutrient medium upon the secretion and properties of pectinase. *Ann. Bot., Lond.*, N.S. **1**, 727.

Menon, K. P. V. (1934). Studies in the physiology of parasitism. XIV. Comparison of enzymic extracts obtained from various parasitic fungi. *Ann. Bot., Lond.*, **48**, 187.

Wood, R. K. S. (1951). The control of diseases of lettuce by the use of antagonistic organisms. I. The control of *Botrytis cinerea* Pers. *Ann. appl. Biol.* **38**, 203.

(*Received* 3 *January* 1950)

MICROBIOLOGICAL CONTROL OF *BOTRYTIS CINEREA* PERS.

II. ANTAGONISM BY FUNGI AND ACTINOMYCETES

By F. J. NEWHOOK

*Imperial College of Science and Technology, London**

Botrytis cinerea has been inhibited by pure cultures of many fungi and actinomycetes on lettuce extract agar and by these and by the mixed flora of soil suspensions on wounded detached lettuce leaves and on wounded leaves of living plants under conditions of high moisture and humidity. Control was exhibited in many cases at 4° C. though this was less marked than that at 15–25° C. Most fungal activity raised the pH to 7·8–8·4 but the use of buffered media showed that most species were antagonistic because of the production of antibiotic substances independently of the high pH. Prior inoculation with soil suspensions was necessary to obtain control of *B. cinerea* on wounded leaves of living plants although such suspensions and several fungi had given control on simultaneous inoculation with *B. cinerea* on wounded detached leaves.

Saprophytic antagonism is responsible for some of the natural control of *B. cinerea* in the field. The amount of available moisture, which in turn is affected by the relative position of the dead tissue on the plant as well as by climatic factors, greatly influences the establishment and growth of *B. cinerea* and the amount of microbiological antagonism likely to be exerted.

Three-weekly applications of suspensions of *Cephalosporium* sp., *Fusarium flocciferum* and *Phoma eupyrena* failed to give a significant increase in the final survival of lettuce in the field. On the other hand, 3-weekly applications of a dust containing tetrachloronitrobenzene gave a highly significant increase in survival, efficiency being greatly increased by the use of cloches.

Earthing up induces early rotting of the petioles of ageing leaves and so probably prevents entry of *Botrytis cinerea* into the stem. Increased survival in hollows as compared with ridges is apparently due largely to this earthing up effect and possibly also to increased microbiological activity on dead tissue under the wetter conditions.

B. cinerea can enter the soil by invasion down the root systems of attacked plants and can attack other root systems in contact with these. Furthermore it has been recovered from the soil over 6 weeks after the disappearance of infected plants from above ground, despite the antagonism shown to be exerted by soil organisms.

Control of *B. cinerea* on *Epilobium* sp., *Ranunculus repens*, *R. flammula* and *Lamium amplexicaule* was obtained by prior inoculation of killed tissue with soil suspensions.

The role of bacterial antagonism to *Botrytis cinerea* and the effect on *Botrytis* attack of the high pH frequently produced by saprophytic organisms on dead lettuce tissue has been discussed in the preceding paper (Newhook, 1951), where the general methods used have also been outlined. The literature on antagonism to *B. cinerea* has been reviewed by Wood (1951).

* Now Mycologist, Plant Diseases Division, Auckland, New Zealand.

Antagonism by fungi and actinomycetes under controlled conditions

Fungal antagonism

During the 1946–7 and 1947–8 seasons over 200 isolations were made from fungi saprophytic on dead lettuce tissue in the field. The most frequent isolates were species of *Fusarium*, *Phoma* (amongst which *Phoma eupyrena* was very common), *Cephalosporium*, *Cladosporium* and several that were non-sporing. *Penicillium* and *Trichoderma* species were notably rare.

(*a*) *Antagonism tests on culture media*

The routine test for antagonism against *Botrytis* was to inoculate the saprophyte under test (referred to hereafter as the antagonist) and *B. cinerea* at opposite sides of a Petri dish and to record the daily positions of the colony margins on the backs of the dishes.

Mutual reaction was conveniently divided into four classes.

(1) Mutual antagonism at a distance.

(2) *Botrytis* antagonized from a distance but the antagonist continuing growth up to or beyond the margin of *B. cinerea* at an unchanged or a reduced rate.

(3) Mutual inhibition on contact.

(4) *Botrytis* inhibited on contact with the antagonist which continued to grow unchanged or at a reduced rate through the *Botrytis* colony.

Neither with any of the fungi tested nor when *B. cinerea* was tested against itself was the *Botrytis* observed to grow into the other colony.

Lettuce extract agar was used for all tests and for most isolates behaviour both on unbuffered and buffered (pH 6·9) media was compared. In both series the pH of the medium at the adjacent edges of the test colonies was measured at the time of inhibition. All except *Geotrichum* and *Botrytis cinerea* itself produced a strongly alkaline reaction which was materially reduced by the advancing *Botrytis* in only a few cases. Many fungi caused stimulation of sporulation of *B. cinerea* at the inhibited colony margin.

As a final test a portion of each of the test colonies was reversed and inoculated with a drop of *Botrytis* spore suspension. After 24 hr. there was no germination with any of the fungi so tested, including *B. cinerea* itself.

Table 1 summarizes the remaining results. It will be seen from the table that in sixteen instances where there was antagonism at a distance on unbuffered agar, buffering reduced the effect to antagonism on contact. On unbuffered agar the majority of *Fusarium* spp. caused inhibition on contact whereas the majority of *Phoma* spp. were antagonists at a distance. This comparison could not be extended to other genera owing to the smaller numbers of isolates available.

(*b*) *Antagonism tests on leaves*

The leaf inoculation technique (Vaseline ring-solid carbon dioxide) described by Wood (1951) was used to test eighty-eight of the isolates from dead lettuce

TABLE 1. *Antagonistic relationship between various strains of fungi and* B. cinerea— *at* 20° C. *on unbuffered* (*U*) *and buffered* (*B*) (pH 6·9) *lettuce extract agar*

Genera or groups of antagonists	Total no. of strains tested	Mutual inhibition at a distance		Antagonist inhibitive at a distance*		Mutual inhibition on contact		Antagonist inhibitive on contact	
		U	B	U	B	U	B	U	B
Fusarium	36	1	.	12 (1)	6 (2)	6	13	17	17
Phoma	13	.	.	11 (5)	5 (1)	.	3	2	5
Cephalosporium	5	.	.	5 (4)	3 (1)	.	1	.	1
Cladosporium	7	.	.	7 (3)	4	.	2	.	1
Non-sporing:									
(*a*) *Dematiaceae*	2	.	.	2	2	.	.	.	.
(*b*) Others	8	.	.	7	4	.	1	1	2
Cylindrocarpon	2	.	.	2 (1)	1 (1)	.	.	.	1
Stemphylium	2	.	.	2	2	.	.	.	.
Penicillium	2	.	.	.	.	2	2	.	.
Alternaria	1	.	.	1 (1)	.	.	.	.	1
Epicoccum	1	.	.	1	1	.	.	.	.
Geotrichum	1	.	.	1	1	.	.	.	.
Trichoderma	1	.	.	1	1	.	.	.	.
Total	81								

* Figures in brackets are the number of antagonists in this section which continued to grow through the *Botrytis* colony.

tissue. These comprised most of the commonly occurring species and included several morphologically similar isolates. The following tests were carried out:

(1) All the isolates were inoculated as spores or mycelium in a drop of sterile water 2 days before *Botrytis cinerea*. Controls received sterile water only instead of the antagonist. Practical considerations demanded that the tests be carried out in three batches, in which there were respectively 7, 8 and 10 replications. Ten strains were duplicated in two of the tests, giving essentially the same results each time. Incubation was at 20° C.

(2) *B. cinerea* was inoculated simultaneously with sixty-one of the antagonists in two batches, with 10-fold replication and incubation at 20° C.

In series (1) some of the test fungi behaved as weak wound parasites and caused the appearance of progressive lesions before the development of *Botrytis* rot in the controls. This occurred particularly with some strains of *Fusarium* and *Phoma*. However, where antagonism occurred later these lesions usually remained quite different in appearance and texture from those in the controls and moreover they failed ultimately to produce *Botrytis* spores. Towards the end of the experiment it was still possible in most instances to carry out a microscopic examination to observe whether the *Botrytis* spores on the still wet inoculation lesions had germinated and penetrated the leaf. As germination and/or penetration was suppressed by many strains in series (1) including most of those associated with progressive lesions it can safely be presumed that the lesions were not caused by *B. cinerea* in these cases. Germination and penetration were invariably good in series (2). Table 2

summarizes the results for series (1). Sporulation was the final criterion for successful *Botrytis* attack.

In general, *Fusarium* spp. tended to suppress germination while *Phoma* spp. were not so effective in this respect, although all the isolates from both genera completely prevented attack by *Botrytis cinerea*. None of the fungi proved to be entirely non-antagonistic and relatively few allowed even a reduced amount of attack by *Botrytis*.

Table 3 summarizes the results for the sixty-one strains of series (2). Fifty-eight of these were strains which had completely prevented attack by *B. cinerea* in series (1). Of this number twenty-eight had allowed some germination while thirty had been responsible for complete inhibition. It was deemed necessary to test three of those strains which had allowed some attack after 2 days prior incubation on the inoculation lesions.

Once again *Fusarium* spp. were in general more effective than *Phoma* spp. From the results of both series it is noteworthy that: (*a*) a large number of common saprophytes is capable of inhibiting *Botrytis cinerea* under conditions optimum for both, the main limiting factor being the relative time of inoculation of the antagonist; (*b*) the results in general and for individual antagonists were very similar to those from tests on lettuce extract agar.

At the conclusion of the experiment pH tests on inoculated leaves of series (1) gave values of 8·0–8·4 in all cases except those inoculated with *Geotrichum* sp. and controls with *Botrytis* alone. In these the maximum pH was 6·8–7·2. Thus the majority of the saprophytic fungi isolated from dead lettuce leaves were responsible for a considerable rise in pH, to a level known to be inhibitory to *B. cinerea* even without the presence of antibiotic substances (Newhook, 1951).

To test the hypothesis that the effect of prior establishment of the saprophyte might be largely one of reduction of available nutrient for *Botrytis*, a further test was made on seven antagonistic strains which, after two days incubation at 20° C., had allowed some germination or attack by *B. cinerea*. Spores of the latter were inoculated in one series with sterile distilled water and in the other with sterile lettuce extract. There was no reduction in inhibition in the second series. The hypothesis, furthermore, cannot explain the many cases where there was complete prevention of germination, as spores of *B. cinerea* will produce germ-tubes over 100 μ long in the absence of any nutrient.

Three of the most frequently occurring saprophytic fungi on dead lettuce tissue in the field, viz. *Phoma eupyrena*, *Fusarium flocciferum* and *Cephalosporium* sp., were tested against *Botrytis cinerea* on wounded detached leaves at 4° C. All three grew well and after 4 days' prior incubation gave complete control of *Botrytis* attack.

Fungi allowing attack by *Botrytis*			Fungi responsible for 100 % prevention of attack by *Botrytis*		
			Botrytis germination allowed		
In > 85 % of lesions	In approx. 50 % of lesions	In approx. 25 % of lesions	In > 50 % of lesions	In < 50 % of lesions	*Botrytis* germination suppressed
Three sets of controls (*Botrytis* alone)	—	—	2 *Fusarium*	2 *Fusarium*	12 *Fusarium*
	—	—	3 *Phoma*	7 *Phoma*	5 *Phoma*
	1 *Cephalosporium*	1 *Cephalosporium*	—	3 *Cephalosporium*	3 *Cephalosporium*
	—	5 non-sporing	4 non-sporing	5 non-sporing	15 non-sporing
	2 *Cladosporium*	2 *Cladosporium*	—	1 *Cladosporium*	2 *Cladosporium*
	—	2 *Cylindrocarpon*	—	—	2 *Cylindrocarpon*
		—	2 *Trichoderma*	1 *Trichoderma*	—
	—	—	1 *Geotrichum*	1 *Epicoccum*	—
	—	—	—	—	4 *Stemphylium*
	—	—	—	—	1 *Alternaria*
	—	—	—	—	2 *Penicillium*
Totals	3	10	12	20	46

TABLE 3. *Results of simultaneous inoculation of* B. cinerea *and saprophytes on lettuce leaves at* 20° C.

Behaviour in Table 2	Number of lesions in which test strains prevented attack by *B. cinerea* (max. 10)					
	0	1–2	3–4	5–6	7–8	9–10
Isolates which had allowed some germination but prevented rot by *Botrytis*	1 *Geotrichum*	1 *Fusarium*	1 *Fusarium*	4 *Phoma*	1 *Fusarium*	1 *Phoma*
	—	1 *Phoma*	1 *Phoma*	3 non-sporing	2 non-sporing	1 *Trichoderma*
	—	1 *Cephalosporium*	1 *Cephalosporium*	—	—	1 *Epicoccum*
	—	1 *Cladosporium*	1 *Cladosporium*	—	—	—
	—	—	4 non-sporing	—	—	—
	—	—	2 *Cylindrocarpon*	—	—	—
	—	—	1 *Alternaria*	—	—	—
	Total 1	Total 4	Total 11	Total 7	Total 3	Total 3
Isolates which had suppressed germination of *Botrytis*	—	1 *Phoma*	1 *Fusarium*	1 *Phoma*	1 *Phoma*	6 *Fusarium*
	—	1 non-sporing	4 *Phoma*	2 *Cephalosporium*	1 *Cladosporium*	2 *Penicillium*
	—	—	1 *Cephalosporium*	1 *Trichoderma*	—	2 *Stemphylium*
	—	—	1 *Cladosporium*	1 non-sporing	—	—
	—	—	3 non-sporing	—	—	—
		Total 2	Total 10	Total 5	Total 2	Total 10
Isolates which allowed some rot by *Botrytis*	—	1 *Cephalosporium*	—	—	—	—
	—	2 *Cladosporium*	—	—	—	
		Total 3				
Totals	1	9	21	12	5	13

Antagonism by Actinomycetes

(*a*) *On culture media*

Actinomycetes were frequently recorded in isolations from leaf debris although they were not evident until several days after the other organisms and appeared to spread on the remainder of the isolation tissue and the media very slowly.

Twenty-three such isolates were inoculated on to unbuffered lettuce extract agar opposite an inoculum of *Botrytis* spores 7 cm. away. Growth margins were recorded daily. Incubation was at 20° C. When growth was static the records in Table 4 were obtained.

TABLE 4. *Records of antagonism by Actinomycetes*

No. of isolations	Width of inhibition zone			Inhibition only on contact
	16–23 mm.	8 mm.	1–4 mm.	
23	13	1	5	4*

* With two of these strains *Botrytis* continued to grow slowly under the Actinomycete colony.

Initial inhibition effects were often obvious 5–6 cm. from the antagonistic colonies. The pH of the medium alongside all actinomycete colonies was 7·6–7·8 and at the margin of the *Botrytis* as further growth was ceasing, 6·1–6·6 (as compared with 5·0 in the controls at the same time), the latter being non-inhibitory values. It was obvious therefore that pH changes were not the main cause of the inhibition.

In the final stages of the experiment a drop of *Botrytis* spore suspension was placed alongside each of the actinomycete colonies. There was good germination alongside the four weak antagonists but none alongside the remaining colonies.

(*b*) *On leaves*

Three strains were tested by the usual technique on leaves in Petri dishes. After 48 hr. prior incubation at 20° C. all completely suppressed germination and attack by *B. cinerea*. On simultaneous inoculation they all allowed germination but controlled attack in nine, six and two lesions respectively out of ten. Similar results were obtained in a second test with the same strains.

ANTAGONISM BY MIXED INOCULA UNDER CONTROLLED CONDITIONS

Soil suspensions

As saprophytic colonization in the field is inevitably by a mixture of large numbers of soil micro-organisms, rain-splashed or airborne, further investigation of the biological control of *Botrytis cinerea* on lettuce seedlings was concentrated on the use of soil suspensions rather than of pure cultures.

(*a*) *On detached leaves*

The Vaseline ring-solid carbon dioxide technique was used as before. From the surface soil of each of four lettuce seedbeds at Slough a suspension was made of

2 g. soil in 10 ml. sterile distilled water. After allowing the coarser particles to settle for 30 sec. the suspensions were decanted off and divided into two portions. Inoculations were carried out in two series:

(1) One portion of each soil suspension was diluted with 3 ml. of sterile distilled water (cf. (2) below). Each inoculation lesion received one drop of suspension, there being twenty-four replications for each of the four suspensions. Twenty-four control lesions each received a drop of sterile distilled water. After 48 hr. incubation at 20° C. each lesion including controls received a drop of *B. cinerea* spore suspension and incubation was continued.

(2) The second portion of each soil suspension was diluted with 3 ml. of *B. cinerea* spore suspension and used for inoculation as before. The twenty-four control lesions each received a drop of *Botrytis* spore suspension of equivalent dilution.

A similar experiment was run at 4° C. using suspensions from the same soil samples. There were twenty-four replications and the interval for prior inoculation in series (1) this time was 4 days.

As with pure cultures of fungi, many progressive lesions developed from the soil inoculations in series (1) at both temperatures before any such rotting began in controls with *Botrytis* alone. Species of *Phoma*, *Fusarium*, *Pythium* and *Cephalosporium* were most commonly isolated from these lesions. As before, appearance, texture and ultimate lack of sporulation indicated that the rot was not due to *Botrytis cinerea*. Direct observations of germination on the lesions with soil suspensions were difficult and inconclusive. Partial inhibition of *Botrytis* was exhibited by many lesions in series (2) at both temperatures: although in the cases concerned there was no sign of attack, *Botrytis* mycelium grew from the drier reverse side of the inoculation lesions, producing occasional conidiophores and also typical appressoria on reaching the glass of the Petri dish. This can be compared with the continued aerial growth and sporulation of otherwise completely antagonized colonies of *B. cinerea* on culture media.

The tests were discontinued when general collapse of the leaves set in. The results, presented in Tables 5*a* and 5*b* show that:

(i) The use of soil suspensions in both series and at both temperatures caused a considerable reduction in the total number of progressive lesions.

(ii) Some of the progressive lesions were caused solely by organisms in the soil suspensions and the records of sporulating *Botrytis* suggest that the degree of control was actually much higher than indicated by comparisons of numbers of lesions, being almost 100 % in all except in the 4° C. series (2) with approximately 70 % of apparent control.

(iii) The figures in the penultimate columns suggest that in series (2) there is little check to the actual establishment of *B. cinerea* but that further attack is prevented soon afterwards.

TABLE 5*a*. *Control by soil suspensions of* B. cinerea *on lettuce leaves at* 20° C.

	No. of progressive lesions (max. 24) after				No. of progressive lesions with sporulating *Botrytis* on 8th day	No. with *Botrytis* restricted to reverse side of inoculation lesion	No. with no visible *Botrytis*
	1 day	2 days	3 days	5 days			
Series (*a*) Control	2	23	23	*	21	1	2
Soil-means	6†	14	16	*	0	1	23
Series (*b*) Control	4	22	24	24	21	2	1
Soil-means	2	14	14	15	0	15	9

TABLE 5*b*. *Control by soil suspensions of* B. cinerea *on lettuce leaves at* 4° C.

	No. of progressive lesions (max. 24) after			No. of progressive lesions with sporulating *Botrytis* on 26th day	No. with *Botrytis* restricted to reverse side of inoculation lesion	No. with no visible *Botrytis*
	16 days	22 days	26 days			
Series (*a*) Control	21	22	*	22	2	0
Soil-means	5	12	*	1	0	23
Series (*b*) Control	19	21	21	20	4	0
Soil-means	7	11	11	7	5	12

N.B. Soil-means = means of the four soil suspensions all of which gave very similar results. The number of days is reckoned from the time of inoculation of *B. cinerea*.

* General collapse of leaves prevented further records at this stage.

† Lesions probably caused by organisms in soil suspension inoculated 2 days prior to *B. cinerea*.

(*b*) *On leaves of living plants*

Plants were established in pots at least 3 weeks before required. In this period naturally infected plants were culled. The Vaseline ring-solid carbon dioxide technique was employed and for economy of space, two to three lesions were made on each plant, one lesion only being on any one leaf. Older leaves were avoided as experience showed that they tended to become moribund during the course of the experiment. Where a lesion progressed so rapidly as to threaten invasion of the stem the particular leaf was removed if it was necessary to continue observations on the remaining leaves.

In one such experiment the following variant treatments were tested:

(1) CO_2 lesion inoculated with *Botrytis*.

(2) CO_2 lesion allowed to dry for 3 days, then inoculated with *Botrytis*.

(3) CO_2 lesion inoculated with soil suspension and *Botrytis* simultaneously.

(4) CO_2 lesion inoculated with soil suspension alone.

(5) CO_2 lesion inoculated with soil suspension, kept moist 3 days, then *Botrytis*.

(6) CO_2 lesion inoculated with soil suspension, kept moist 3 days, allowed to dry for 3 days, then inoculated with *Botrytis*.

In an earlier test in which the carbon dioxide lesions were kept moist for 3 days before inoculation with *Botrytis*, airborne contamination with *B. cinerea* caused early infection in most lesions so that treatment (1) was maintained as the sole control in this experiment. All the soil inoculations were done at the same time, variation being confined to the time of inoculation of the *B. cinerea* suspensions which were made from sporing cultures of equivalent age. As dead tissue in the field is subject to alternate periods of wetting and drying, treatments (2) and (6) were included. Except for the periods of drying, treatments were carried out in a glass-topped moist chamber. The external (greenhouse) temperature ranged from 11° to 20° C. (av. 18°). There were twenty-four replications for each treatment. Results are presented in Table 6.

TABLE 6. *Effect of soil suspensions under different conditions, on attack by* B. cinerea *from inoculation lesions on lettuce leaves (average temperature* 18° C.)

Treatment	Inoculation procedure and total no. (out of possible 24) of progressive lesions on the days indicated								No. of lesions with *Botrytis* attack*
	1st day	5th	8th	10th	12th	14th	16th	19th	
1	—	Kill† + *Botrytis*	0	23	23	23	23	23	23
2	—	Kill, dry 3 days	+ *Botrytis*	13	23	24	—	—	24
3	Kill + soil + *Botrytis*	14	22	23	24	—	—	—	20
4	Kill + soil	0	2	5	7	12	20	21	3
5	Kill + soil	+ *Botrytis*	2	3	3	10	16	19	1
6	Kill + soil	Dry 3 days	+ *Botrytis*	6	6	16	22	23	8

* Indicated by penetration of petiole and stem and by sporulation.
† Centre of ring killed with solid carbon dioxide.

Table 7 summarizes the data from the experiment just described and from four others run at average temperatures of 15°, 9°, 19° and 20° C. respectively. The figures given are counts of successful *Botrytis* attack. Progressive lesions associated with mildly parasitic soil organisms sometimes developed as before, but they seldom penetrated the stem and *Botrytis* spores never appeared. Treatments (1)–(6) were as described above. Treatment (7) was equivalent to treatment (5) except that the Vaseline ring was omitted in the initial stages. Under these circumstances the inoculation drops did not stand for long on the lesions so these were re-wetted daily with sterile distilled water. Before inoculation with *B. cinerea* a partial Vaseline ring was applied in order to retain the inoculum. In treatment (8) the soil suspension was incubated for only one day prior to inoculation of *B. cinerea*.

The following conclusions are drawn from a study of the results presented in Tables 6 and 7.

(*a*) There was no significant reduction in *Botrytis* attack with treatments (2)

TABLE 7. *Summarized results of five experiments on the control of* B. cinerea *by soil suspensions*

	No. of lesions with successful attack by *B. cinerea*				
Treatment	Exp. 1, 18° C. Max. 24	Exp. 2, 15° C. Max. 30	Exp. 3, 9° C. Max. 30	Exp. 4, 19° C. Max. 19	Exp. 5, 20° C. Max. 15
1	23	26	30	19	15
2	24	27	—	—	—
3	20	—	30	19	15
4	3	3	1	—	—
5	1	4	4	4	1
6	8	9	—	—	—
7	—	12	—	—	—
8	—	—	—	4	5

and (3). The latter is in strong contrast to the almost complete control given by simultaneous inoculation of soil and *Botrytis* on detached leaves.

(*b*) The examples of *Botrytis* attack in treatment (4) with soil alone were probably due to the airborne contamination with *B. cinerea* known to be present, as shown by exposed plate tests.

(*c*) Three days prior incubation of soil suspensions (treatment 5) gave almost complete control of *B. cinerea*, while one day prior incubation (treatment 8) gave a considerable degree of control.

(*d*) In treatment (6), although there was a large measure of control, drying out reduced the efficiency of inhibition by the soil suspensions.

(*e*) The less efficient method of prior inoculation (treatment 7) where the Vaseline ring was omitted during first stage of the test was reflected in the lower degree of control (though still 60 %).

(*f*) There was close agreement between the same treatments repeated in the different experiments, despite the differences between soil samples, temperatures and other experimental conditions.

With the Vaseline ring-solid carbon dioxide technique, complete control of *B. cinerea* on lettuce seedlings by prior incubation of loam and compost suspensions has since been obtained on two occasions at Auckland, New Zealand.

Airborne inocula of saprophytes

Plants with a total of thirty carbon dioxide lesions of the usual type were left on the open greenhouse bench. No inoculum was added but lesions were maintained wet with sterile distilled water for 5 days before the plants were transferred to a moist chamber. By this time *Botrytis* attack had begun to appear and by the 14th day it was present on all lesions. During the first period of the experiment two plates, one beef-peptone and one lettuce extract, were exposed for 2 hr., yielding respectively

114 and 47 bacterial colonies and 194 and 204 fungal and actinomycete colonies. The latter (204) consisted of

Penicillium spp.	172	*Fusarium* and *Cephalosporium* spp.	5
Cladosporium spp.	10	Miscellaneous	7
B. cinerea	5	Actinomycetes	1
Trichoderma sp.	2		
Pullularia sp.	2		

Thus, under this set of greenhouse conditions at least, as soon as dead tissue appears, despite the fact that *Botrytis cinerea* was in a minority in the natural deposition of spores, there was nevertheless *Botrytis* attack in all cases. Presumably therefore, the conditions approximated to those of the earlier experiments in which there was simultaneous inoculation of *Botrytis* and a mixture of saprophytes.

Natural antagonism in the field

pH *of dead tissue*

Comment has been made in a preceding paper (Newhook, 1951) on the frequency with which a high pH (7·8–8·4) has been found in association with dead tissue in the field. From the evidence in previous sections a high pH may be taken as a reliable indication of much saprophytic activity with associated antagonism to *B. cinerea*.

Evidence from distribution of Botrytis *on seedlings*

In the 1946–7 season no *B. cinerea* was seen in outdoor seedbeds at Slough until 4 February (except in one plot where it followed severe damping-off caused by *Rhizoctonia solani*). At this time all seedlings showed up to six or eight dead or yellow outer leaves together with a considerable amount of frost damage on other leaves. *Botrytis cinerea* was isolated from frost lesions but not from older dead leaves, on which there was much general saprophytic growth. This restriction of *Botrytis* to frost-damaged leaves was observed consistently up till the time of transplanting, 2 June, on large numbers of randomly chosen seedlings tested by the humidity dish method described earlier (Newhook, 1951). Table 8 gives the results for one such

Table 8. *Number of leaves of different ages showing infection with* B. cinerea *after four days in humidity dishes*

Leaf no.	1	2	3	4	5	6	7	8	9	10	11	12	13	Total no. of infected leaves
No. of infected leaves (max. 24)	0	0	0	1	6	6	16	15	14	9	6	4	2	79

test on twenty-four plants which had showed no outward sign of *Botrytis* in the field, and summarizes the distribution of *Botrytis* according to the relative age of the leaves, the oldest being referred to as no. 1 and the bud no. 13. Nos. 1–4 and sometimes also 4–6 were rotting remains of leaves or merely petiole stumps. These were followed by frost-damaged outer leaves, younger green leaves and the bud.

It will be seen that *Botrytis* was consistently absent from well-rotted leaf remains but was commonly present on frost-damaged leaves. Part of the difference would no doubt be due to the relative differences in area offered for establishment of *B. cinerea* but with such a high percentage infection per plant (100 %) and the more favourable moisture conditions about the bases of the plants, this cannot be the only explanation. From the evidence in the previous sections it is concluded that saprophytic activity on the older dead leaves had given protection against *Botrytis*. By the 14th day of the test all signs of *Botrytis* had disappeared. Bacteria and protozoa were dominant in the debris and due no doubt to antagonism and lysis five plants remained free from any further attack by *B. cinerea*.

The above results were confirmed repeatedly in humidity dish tests, although in general the percentage infection by *B. cinerea* was lower. The results were similar but less constant in the drier season 1947–8.

Colonization of frost lesions by B. cinerea *and saprophytes*

(1) On 3 December 1947 severe frosts caused much damage to seedlings at Slough, in the form of large central lesions on horizontal leaves and marginal scorch on others. The former were regarded as suitable material for following the progress of infection and inhibition of *B. cinerea*.

Rain fell on each of the 5 days succeeding 3 December, splashing all plants with soil. From then on there was fine weather with drying winds with a few light showers on 18 and 19 December. However, central lesions were usually dry again within an hour of rain. On 8, 17 and 23 December a random selection of leaves with central lesions was taken to the laboratory. The lesions, surrounded by green tissue, were placed on non-nutrient agar. Twenty-four such lesions were inoculated on 8 December with a suspension of *B. cinerea* spores and incubated for 24 hr. at 20° C. All showed 100 % germination. On 17 and 23 December the lesions were separated into two groups, to be incubated at 20° and 4° C. respectively. Within each group some lesions were inoculated immediately and the remainder after 24 hr. incubation. Their behaviour is shown in Table 9. The figures in the table give the numbers of

TABLE 9. *Effect of inoculating leaves suffering from frost lesions*

		December 17				December 23			
			Germination (%)				Germination (%)		
Inoculation	Temp. (C.)	Total	60–100	10–50	0–10	Total	60–100	10–50	0–10
Immediate	20°	12	12	0	0	13	8	3	2
	4°	12	12	0	0	7	1	2	4
After 24 hr.	20°	12	0	1	11	7	0	0	7
	4°	12	3	7	2	7	0	2	5

lesions which showed the degree of germination recorded at the head of the corresponding column. The results show that:

(*a*) There was a tendency for a reduction in germination of the inoculum as the frost-damaged leaves were left longer in the field.

(*b*) Incubation under moist conditions for 1 day accentuated the reduction and this effect, though less at 4° C., was nevertheless clearly shown at this lower temperature.

(*c*) Six days under relatively dry field conditions were not as effective in bringing about this reduction in germination as 1 day's incubation under continuous moist conditions.

Thus although saprophytic establishment was relatively slow on the easily desiccated lesions of the type investigated, it was apparently sufficient by 23 December to inhibit considerably the germination of *B. cinerea*.

(2) There was natural infection by *Botrytis* on twenty-two out of thirty-four lesions plated on 23 December and incubated at 20° C. There was a strong inverse correlation between the presence of obvious saprophytic growth and *B. cinerea* in these cases, as illustrated by Table 10. *Botrytis* growth on to the surrounding tissue and out on to the medium was confined, also, to regions apparently not colonized by other fungi.

TABLE 10. *Correlation between* Botrytis *infection and visible saprophytic growth*

	No. of lesions with *B. cinerea*		
	Dominant	Weak	Absent
Much visible saprophytic growth	0	9	12
Little visible saprophytic growth	10	0	0
No visible saprophytic growth	3	0	0

From 215 whole or part frost lesions on lettuce seedling leaves plated on to plain agar from December to March the following fungi were recorded:

(*a*) *B. cinerea* 130 isolations.

(*b*) Species of *Cladosporium* 205, actinomycetes 125, *Fusarium* 110, *Phoma* 70 and *Pullularia* 61 represented the earliest, commonest and usually dominant saprophytes.

(*c*) *Alternaria* 85 and *Stemphylium* 30 tended to be later in appearance together with 47 miscellaneous isolates that were not identified.

(*d*) *Penicillium* 5, *Trichoderma* 3 and *Aspergillus* 2 were notably infrequent in occurrence.

(*e*) Although *Pythium* 8 and *Geotrichum* 4 were amongst the most frequent isolates from soil immersion tubes of the type described by Chesters (1948) used in the seedbeds throughout the season, they occurred only rarely on frost lesions.

Botrytis *in the soil*

Although so many soil organisms have been proved to be antagonistic to *B. cinerea* and even to cause lysis of active mycelium, *Botrytis* can behave as a successful soil invader and remain viable, under certain circumstances at least, for some weeks.

It was frequently observed sporing on the debris of plants it had killed, below the surface of the soil. During the winter 1947–8, at Slough, 472 soil-immersion tubes were planted beside or below healthy and diseased lettuce seedlings and *B. cinerea* was recovered from seventy-nine tubes, seventy-four of which had been inserted below seedlings killed some time previously by *Botrytis*. The remaining five were within 2 in. of groups of such dead plants, so that in all cases the *Botrytis* seemed to be a soil invader by way of the roots of infected seedlings. *B. cinerea* was still isolated from marked positions over 6 weeks after disappearance of the dead plants from the surface.

Further work on the pathogenicity and longevity of *B. cinerea* in the soil is obviously required. Soil invasion by attack down the substantial lettuce root system is understandable despite the antagonism shown earlier to be exerted by soil organisms. The initially acid reaction accompanying *Botrytis* attack might be of importance in delaying bacterial action and moisture conditions would probably be of great importance in determining the course of microbiological activity, including lysis of the *Botrytis* mycelium.

FIELD TRIALS TO CONTROL *B. CINEREA*

(1) In the seasons 1946–7 and 1947–8, at Slough, extensive trials were carried out on seed-bed lettuce to determine the effect of earthing up on the severity of *B. cinerea* attack. In the latter season also, spore and mycelial suspensions of three fungi, *Cephalosporium* sp., *Fusarium flocciferum* and *Phoma eupyrena*, which had proved to be antagonistic to *Botrytis* on leaves at 4° C. were sprayed on to plants at 3–4-week intervals. For comparison, in both seasons, 3–4-weekly applications were made to other rows with a dust containing tetrachloronitrobenzene, the effectiveness of which against *B. cinerea* had been shown earlier (Brown & Montgomery, 1948). Treatments in the second season were carried out on seed-beds in the open and under cloches and most of the treatments were repeated on open seed-beds sown on three dates, viz. 19, 25 and 30 September. There were respectively eleven, thirteen and eleven replications of 10 ft. rows per treatment with a germination rate of over 200 per row.

In the first season earthing up increased survival by 43 % ($P = 0{\cdot}05$) while dusting gave an increase of 74 % ($P = 0{\cdot}01$) over the controls. In the second season there was no significant increase in survival due to earthing up, possibly because of complications arising from activity of *Rhizoctonia solani*. Dusting increased survival by 180 % under cloches ($P = 0{\cdot}001$) and 64, 69 and 30 % respectively for the three dates of sowing in the open ($P = 0{\cdot}001$, 0·01 and 0·01), with a further increase in survival after transplanting of 110 % for seedlings originally under cloches ($P = 0{\cdot}001$) and 50, 17 and 5 % for seedlings overwintering in the open ($P = 0{\cdot}001$, 0·05 and N.S.). *Fusarium flocciferum* gave no control. *Cephalosporium* sp. gave a just significant reduction in infection (39 %: $P = 0{\cdot}05$) early in the season but this was not maintained. With the *Phoma eupyrena*-treated seedlings there was a slightly significant

increase in survival (20 %: $P=0{\cdot}05$) with the late sowing but the increase (17 %) for the early sowing was not significant. At the time of transplanting all organisms were recovered regularly from dead leaf tissue 8 days after the final application.

(2) In autumn-planted open plots in the second season dusting gave an increased survival of 42 % ($P=0{\cdot}001$); *P. eupyrena* (16 %) and earthing up (13 %) gave no significant difference from the controls, nor did *Cephalosporium*, *Fusarium flocciferum* and shallow and deep planting. There were seventeen replications with a total of 6320 plants.

(3) The results of a further seed-bed trial in which lettuce was sown on ridges and in hollows confirmed those recorded by Brown & Montgomery (1948). There were three widely separated plots with a total of fifteen replications, within each of which four rows were sown normally on the flat, three were on 1 ft. high ridges and two were in the separating hollows. As germination on the ridges in the dry autumn was uneven, the two outer rows on the flat were converted into low ridges one month later by lateral excavation. There was almost 100 % mortality on one plot and part of a second. Survival counts on lifting early in March were not analysed owing to the great differences in germination between treatments, but equal numbers of plants per row from the original plots were planted out (total 3540) and survivals recorded early in May (Table 11).

TABLE 11. *Percentage survival on 5 May reflecting the earlier seedbed conditions*

Treatment	Hollows	Flat	Secondary ridges	Original ridges
Survival means	74	68	60	41
Probability level	0·01	0·01	0·001	

The stems of plants lifted from the hollows were almost invariably devoid of dead leaves as compared with those on the ridges. The latter also had more stem lesions. Plants on the flat and on the secondary ridges were intermediate in these respects. Thus, one of the main reasons for the reduction in infection would appear to be the severance of the petioles of older leaves from the stem by organisms in the soil building up about the bases of the plants in the hollows, i.e. an indirect form of microbiological control of *Botrytis* attack. Attack by *Marssonina* was more severe in the hollows, indicating wetter conditions. Coverage of plants by rain-splashed soil particles was also much greater in the hollows than elsewhere so that there may well have been increased microbiological action on dead leaf tissue in the hollows. Soil temperatures at a depth of 1 in. on the ridges were seldom more than 1° C. lower than in the hollows. However, the roots of plants on the ridges would have been more generally exposed to frost injury and this may have rendered these plants more susceptible to infection. The relative importance of the above factors could not be estimated.

Microbiological control of *B. cinerea* on plants other than lettuce

The strain of *B. cinerea* used in the work described was pathogenic after wounding to certain weed species occurring commonly in the seed-beds at Slough, viz. *Senecio vulgaris*, *Lamium amplexicaule*, *Chenopodium album* and *Stellaria media*. *Capsella bursa-pastoris* was not susceptible. Certain species growing in woodland bogs where the more or less constant moisture should be favourable for the growth of *Botrytis* and saprophytes in general were also susceptible, viz. *Epilobium* sp., *Ranunculus repens* and *R. flammula*.

Using the Vaseline ring-solid carbon dioxide technique, prior incubation of soil suspensions on the lesions for 5 days gave the following degrees of control of *Botrytis cinerea* on four of the above species so tested: *Epilobium* sp., 100%; *Ranunculus flammula*, 100 %; *R. repens*, 46% ; *Lamium amplexicaule*, 60 %.

Although the leaves of the two *Ranunculus* species were susceptible to *Botrytis*, attack was never observed to progress further down the slender petioles than to just above soil level. It is not inconceivable that microbiological control might have taken place under these conditions as contrasted with the continuance of *Botrytis* attack down the relatively stout petioles, stems and roots of lettuce.

Discussion

Experiments under controlled conditions have shown that *B. cinerea* can be prevented from attacking lettuce and to a certain extent other plants by the majority of saprophytic fungi, actinomycetes and bacteria occurring on dead lettuce tissue in pure or in mixed culture, provided the following conditions obtain:

(1) A reasonable period for establishment of the saprophytes before inoculation with *Botrytis*.

(2) Actual moisture or a high humidity.

(3) Moderate temperatures, although some organisms can still control *B. cinerea* to a high degree at 4° C.

Thus, for the soils tested at least, the general antagonism to *B. cinerea* developed by the organisms of the soil suspension was not reduced below an effective level by any considerations of mutual antagonism between saprophytes, lability or adsorption of antibiotic substances, etc. This is important from the point of view of control under natural conditions since the soil is almost certainly the main source of inoculum for the colonization of dead tissue in the field.

Most bacterial and fungal activity raised the pH of media and dead lettuce tissue to 7·8–8·4, a level which is itself inhibitory to growth of *B. cinerea* and to the activity of any pectinase produced. However, the use of buffered media indicated that most species were antagonistic because of the production of antibiotic substances independently of the high pH. In any case the high pH would provide an alternative as well as an additional inhibitory system in instances where the antibiotic

substances were labile or were destroyed by other organisms or by substances in the substrate.

The available comparisons between antagonism tests on lettuce extract agar, detached leaves and leaves on living plants show that there is often a considerable amount of similarity in the behaviour of any one organism or soil suspension on the different substrates. Similar pH changes occur and similar relative amounts of antagonism are often shown. Differences in behaviour between the sets of conditions are usually of degree, with antagonism decreasing from tests on media to tests on plants. Some of the considerations discussed earlier (Newhook, 1951) in relation to bacterial colonization of dead tissue as compared with behaviour on media may apply equally to fungal establishment.

Simultaneous inoculation of *B. cinerea* and soil suspensions on detached leaves gave 100 % control, yet there was no control of *Botrytis* by similar simultaneous inoculations on wounded attached leaves of living plants. The difference in behaviour may have been due largely to differences in experimental conditions, e.g. humidity and free moisture beyond the inoculation lesions were greater in the Petri dishes than in the imperfectly sealed moist chambers used for most of the tests on living plants. Arrest of attack that had already started was frequently observed in the humidity (Petri) dish tests. In the field thoroughly wet conditions accompanied by much saprophytic inoculum, e.g. about the bases of plants in wet weather or during snow-cover and thaws and with deep-planting or earthing-up in wet soil, may thus account for some of the many observations of cessation of attack by *Botrytis*, first described by Abdel-Salam (1934) as healing of stem lesions. It is suggested as a hypothesis that the healing may follow antagonism of *B. cinerea* by soil organisms under wet conditions.

Several possible explanations are offered for the failure to obtain a significantly increased final survival in the field after regular application to seedlings of suspensions of fungi which had given satisfactory inhibition of *Botrytis* under controlled conditions:

(1) It is possible that *B. cinerea* can attack yellowing leaves not yet suitable for colonization by saprophytes and so initiate attack before antibiotic control is possible.

(2) The amount of biological activity resulting from natural colonization by saprophytes was already as great as the prevailing conditions of moisture, temperature, etc., would allow, and was not likely to be increased materially by such artificial inoculation at 3–4-weekly intervals.

(3) By virtue of its parasitic nature, enabling it to obtain moisture from the tissue being attacked by the pectinase produced, *B. cinerea* may have had a greater tolerance of desiccation than many of the saprophytes used and antibiotic production by the latter may have been slow under conditions of low moisture. This is supported by the observations on frost lesions of a type which is liable to rapid drying out again after rain or dew.

(4) The chances of successful prior establishment of saprophytes before *Botrytis* infection are greatly reduced during the season as the number of sporulating centres of *Botrytis* increases. Part of the efficiency of dusts containing penta- or tetrachloronitrobenzene is due to the fact that they suppress sporulation of *B. cinerea*.

(5) Alternation of dry and wet conditions, as was shown in a greenhouse experiment, reduces the efficiency of biological antagonism. Such an alternation is, of course, normal in the field.

In summary therefore it may be stated that microbiological control of *B. cinerea* on lettuce and other plants can be produced under controlled conditions and that it does occur under natural conditions on seedlings overwintering in the field.

The writer wishes to express his thanks to Professor W. Brown, F.R.S., for suggesting the subject for this research and for helpful criticism during its course; and to Professor C. G. C. Chesters for discussions and advice on the use of soil-immersion tubes.

The work was carried out during the tenure of a New Zealand Government Rehabilitation Bursary.

REFERENCES

ABDEL-SALAM, M. M. (1934). *Botrytis* disease of lettuce. *J. Pomol. Hort. Sci.* **12**, 15.

BROWN, W. & MONTGOMERY, N. (1948). Problems in the cultivation of winter lettuce. *Ann. appl. Biol.* **35**, 161.

CHESTERS, C. G. C. (1948). A contribution to the study of fungi in the soil. *Trans. Brit. mycol. Soc.* **30**, 100.

NEWHOOK, F. J. (1951). Microbiological control of *Botrytis cinerea* Pers. I. The role of pH changes and bacterial antagonism. *Ann. appl. Biol.* **38**, 169.

WOOD, R. K. S. (1951). The control of diseases of lettuce by the use of antagonistic organisms. I. The control of *Botrytis cinerea* Pers. *Ann. appl. Biol.* **38**, 203.

(*Received* 29 *March* 1950)

THE CONTROL OF DISEASES OF LETTUCE BY THE USE OF ANTAGONISTIC ORGANISMS

I. THE CONTROL OF *BOTRYTIS CINEREA* PERS.

By R. K. S. WOOD

Imperial College of Science and Technology, London, S.W.7.

(With 1 Text-figure)

The antagonism of a number of bacteria, actinomycetes and fungi to *Botrytis cinerea* in pure culture on lettuce extract agar has been investigated. A considerable number were antagonistic at 25° C., a few showed antagonism at 15° C. and a limited number grew and were active at 5° C.

Certain of these organisms were able to control rot of detached lettuce leaves when inoculated before or simultaneously with *B. cinerea*. The latter is unable to penetrate areas of dead tissue colonized by selected antagonists.

Control of rot was also obtained when leaves of growing plants were similarly inoculated.

Substantial control of disease occurred when young potted plants in frames were sprayed with suspensions of selected antagonists in 1·0 % glucose solution.

The practicability of this method of control is discussed.

In the cultivation of spring and winter lettuce, the stand of seedlings in frames and open seed-beds is often seriously reduced by disease during the winter. Greatest loss is caused by the activity of *Rhizoctonia solani* Kühn and *Botrytis cinerea* Pers. The former is responsible for a typical post-emergence damping-off while the latter invades moribund or damaged tissue from which the infection spreads, the seedling dying after invasion of the main axis. The relative importance of these two pathogens has still to be accurately assessed, but it seems likely that while *Rhizoctonia* is responsible for some loss in early winter, *Botrytis* causes the much greater losses which occur later. The importance of the earlier though much less serious attack by *Rhizoctonia* is probably in the production of patches of dead plants which after colonization by *Botrytis* produce a large spore-inoculum available for widespread infection of moribund and frost-damaged tissue later in the winter.

While substantial control of *Botrytis* attack may be obtained by the use of proprietary fungicides containing pentachloronitrobenzene, the observations of Brown & Montgomery (1948) in connexion with winter seed-beds suggested another method. It was shown that survival of seedlings in hollows was significantly higher than on ridges or level ground. The reasons for this were not immediately apparent but it was suggested that it might have been caused by the covering of leaf bases by soil washed down from the sides of the ridges leading to their rapid colonization by soil saprophytes and exclusion of *Botrytis*. With this in mind, work was begun in 1946 with

the object of investigating the possibility of controlling *Botrytis* attack by the direct use of antagonistic organisms. The complementary aspect of the problem, viz. the extent to which disease caused by *B. cinerea* is controlled under natural conditions by saprophytic activity was investigated during 1947 and 1948 by Newhook (1951*a*, *b*).

The activity of saprophytes in the control of plant disease is an aspect of the frequently observed phenomenon of 'antagonism' between micro-organisms using this word in its widest sense to describe any activity of one organism, the antagonist, which significantly reduces or adversely modifies the growth of another.

Antagonism to *Botrytis* spp. in pure culture has been reported by a number of workers. Thus Porter (1932) showed that the development of three species was inhibited in the presence of *Bacillus mesentericus vulgatus* and another unidentified bacterium.

Two bacteria causing lysis of the mycelium of *Botrytis cinerea* were reported by Khudiakoff (1935) to be of widespread occurrence in different soils. Asthana (1936) showed that cultures of *B. cinerea* on plates of lettuce extract agar were confined to small areas by a number of fungi and an unidentified bacterium. Similar effects were observed by two of six species of actinomycetes tested by Alexopoulos & Herrick (1942), while Brian & Hemming (1945) have used spores of *B. allii* in estimating the antibiotic properties of filtrates derived from liquid cultures of a wide range of fungi.

A certain amount of further work relates to the activity of saprophytes in controlling disease of aerial parts of plants. Fawcett (1912) simultaneously inoculated walnut branches with *Pseudomonas juglandis* and *Dothiorella gregaria*. No lesions were produced with the mixed inoculum but large cankers result when the fungus alone was used. Savastano & Fawcett (1929) noted that *Botrytis cinerea* reduced rot of lemon fruit caused by *Penicillium* spp. and (1930) showed that various species of *Fusarium* inhibited invasion of the wood of citrus trees by *Deuterophoma tracheiphila*. In somewhat similar studies Vasudeva (1930) reported nine fungi which reduced fruit-rot of apple resulting from inoculation with *Monilia fructigena*. Bamberg (1931) isolated a number of bacteria which, simultaneously inoculated with sporidia of *Ustilago zeae*, largely prevented infection. Koch (1934) investigated the consistent appearance of *Trichothecium roseum* on the conidial stroma of *Dibotryon morbosum*. He considered the former to be parasitic and responsible for some natural control of the pathogen. The saprophyte was established on stromata by inoculation; its subsequent growth destroyed immature perithecia. Asthana (1936) was able to reduce rotting of lettuce leaves by *Botrytis cinerea* by addition of a number of fungi to the inoculum. Hendrickx (1937) showed that the development of *Ophiostoma ulmi* could be inhibited by a bacterium isolated from diseased elm branches. Matsumoto (1939) was able to prevent infection of the leaves of *Eichhornia crassipes* by addition of spores of *Trichoderma lignorum* to an inoculum of *Hypochnus sasakii*. More recently, Newhook (1951*a*, *b*) has shown that a considerable number of organisms occurring saprophytically on dead leaves of lettuce were antagonistic to

Botrytis cinerea in pure culture and were able to control rot of leaf tissue under conditions approximating to those occurring naturally.

It is apparent from the above that a fairly wide range of organisms have been reported antagonistic to *B. cinerea* or related species and that a number of workers have been alive to the possibility of controlling disease of aerial parts of plants with antagonistic organisms. Rarely, however, has control under conditions approaching those found naturally been reported. An attempt to do so has been made in the work recorded below.

General experimental methods

The broad plan of the experimental work was as follows. The antagonism to *Botrytis cinerea* of a wide range of organisms was studied in pure culture over a range of temperatures. On the basis of these studies, a selection was made of organisms with properties thought to be best suited for the ends in view. These included, the ability to act antagonistically to *B. cinerea* over a wide temperature range and under nutrient conditions approximating to those at the surface of a damaged lettuce leaf, rapid growth and/or profuse sporulation. Further tests were then made to see if these organisms inhibited rot of detached leaves and petioles under controlled conditions in the laboratory. Finally, tests were made with a limited number of organisms under frame conditions.

Throughout the work, a single isolate of *B. cinerea* from a diseased lettuce plant was used. Spore suspensions were used for all inoculations and prepared from week-old cultures on potato-extract agar by removing the spore-bearing mat, shaking well in sterile liquid and straining through sterile muslin to remove the larger hyphal fragments. The densities of these suspensions were adjusted by addition of sterile liquid. Media and inocula were prepared with a lettuce extract obtained by adding 5·0 % dried lettuce leaf to tap water, boiling for 10 min., sieving through muslin, filtering under pressure and making up to the original volume. The same stock of leaf was used throughout the work.

Leaves or seedlings used in experiments on disease control were obtained from frame or seed-bed sowings of the varieties 'Imperial' or 'Trocadero'.

The organisms used came from a number of different sources. Some were isolated from soil suspensions, others first appeared as contaminants in discarded plate cultures of *B. cinerea*, and free use was made of those previously reported antagonistic to this or other pathogens. Attention has been mainly confined to spore-forming bacteria, actinomycetes and penicillia, since past work had shown that these groups contain a higher proportion of antagonists than others.

The most striking antagonistic effects in pure culture were shown by the following:

I. Bacteria. *Bacillus subtilis*, *B. brevis*, *B. dendroides*, *B. anthracoides*, *B. megatherium*, *Pseudomonas fluorescens*, *Ps. aeruginosa*.

II. Actinomycetes. *Streptomyces violaceus*, *S. albus*, *S. antibioticus*, *S. lavendulae*, two unidentified *Streptomyces* spp. and Meredith's actinomycete.
III. Penicillia. *Penicillium clavariaeforme*, *P. patulum* and other unidentified spp.
IV. Other fungi imperfecti. *Trichoderma viride* and *Fusarium* spp.

Antagonism on agar media

A number of experiments were carried out using lettuce extract agar, only two of which will be reported in detail. In the first plates were inoculated at the centre with *Botrytis cinerea* and at the periphery with one of the selected antagonists. These, being often slow growing, were sown in three areas each 20 × 10 mm. spaced equidistantly 30 mm. from the centre. Suspensions in sterile distilled water of spores or hyphal fragments of actinomycetes and fungi were used. With bacteria it was necessary to avoid free liquid on the agar surface as far as possible so inoculations were made directly from slope cultures. Even so, many forms spread rapidly over the surface of the agar, making it impossible to obtain comparative values of their antagonism.

After inoculation, plates were incubated at 5°, 15° or 25° C. The growth rate of the antagonists varied considerably, even at optimum temperatures. Thus, while the majority of bacteria, actinomycetes and penicillia grew little from the inoculum, fungi grew rapidly towards the centre of the plate. The behaviour of the *Botrytis* mycelium in the vicinity of the antagonist also varied. In some cases it grew through the colony of the antagonist with no indication of inhibition. In others, although apparently unaffected before the colony, no penetration occurred. Frequently, however, growth stopped some distance away from the antagonist to give a distinct zone free of mycelium. The hyphae of *Botrytis* bordering this zone were often discoloured and malformed. The malformation was sometimes most pronounced and characteristic of a specific antagonist.

In general, the best picture of the antagonistic properties of the various organisms tested is given by the amount of radial growth of *Botrytis* towards the antagonist. In Table 1 a radial growth of >29 mm. is taken to indicate little or no antagonism: 25–29 mm. slight antagonism; 15–24 mm. pronounced antagonism and <15 mm. very pronounced antagonism. In certain instances bacterial antagonists spread rapidly over the surface of the agar, completely inhibiting the growth of *Botrytis*. This action is recorded as 'envelopment'. The results for each of the four groups of antagonists previously listed are shown together.

Bearing in mind the fact that the organisms tested were not selected at random but with an eye to their future use in disease control, the following are the general conclusions from the data. A considerable proportion of the bacteria, actinomycetes and penicillia were highly antagonistic at 25° C. This activity decreased sharply at 15° C. while at 5° C., with the exception of certain penicillia, antagonism was absent, no doubt because of the very limited growth at this temperature. A few penicillia showed quite striking antagonism at all temperatures. In these three

groups the antagonistic effects almost always took the form of a 'zone' about the colony free of *Botrytis* hyphae.

TABLE 1. *Tests of antagonism on lettuce extract agar*

Degree of antagonism	Radial growth of *B. cinerea* (mm.)	Group I (bacteria)			Group II (actinomycetes)			Group III (penicillia)			Group IV (fungi imperfecti)	
		5°	15°	25°	5°	15°	25°	5°	15°	25°	5°	15° (C.)
None	>29	32	11	2	11	3	—	13	—	—	9	—
Slight	25–29	—	2	—	—	5	5	3	12	2	3	5
Pronounced	15–24	—	8	17	1	1	6	4	6	7	3	6
Very pronounced	<15	—	—	2	—	2	4	—	—	—	—	2
Doubtful	Enveloped	—	12	7	—	—	—	—	—	—	—	—
Total tested		32	33	28	12	11	15	20	18	9	15	13

With *Trichoderma viride* and *Fusarium* spp., although antagonism again increased with temperature, it was still quite pronounced in a number of cases at 5° C. Generally, 'zones' were absent. The fungi grew rapidly towards the advancing hyphae of *Botrytis*. When within 1–2 mm. of each other, further growth of both stopped in the majority of cases. In others, growth of *Botrytis* stopped while that of the antagonist continued.

In very few cases was there any suppression of sporulation, even when antagonism was most pronounced. On the contrary, a frequent feature was the increased sporulation along the leading edge of the colony as it approached the antagonist.

In the second series of experiments, competition between inocula was investigated. An area 15 mm. square in the centre of the plate was inoculated with 0·025 c.c. of suspensions of selected antagonists or with a 1·0 % suspension of seed-bed soil. After incubation for periods which differed with the temperature, 0·025 c.c. of *Botrytis* spore suspension containing approximately 2500 spores was added to the centre of the inoculated square and spread to occupy an area approximately 10 mm. square. To escape from the antagonist it was thus necessary for the *Botrytis* spores to germinate and grow through an area occupied by spores or young colonies of the antagonists.

In the majority of cases the results took one of two forms. Either the growth of *Botrytis* (recorded as +) covered the plate sporulating freely or the growth of the antagonist (recorded −) completely inhibited that of *Botrytis*. In some cases the former effect was doubtless caused by the poor growth of the antagonist. In others, owing to the more rapid growth at first, *Botrytis* itself acted antagonistically. In a minority, intermediate effects were observed (recorded as + −). The antagonist grew well, but *Botrytis* was able to grow through the colony in its early stages and grow freely on the surface of the agar not occupied by the antagonist.

The antagonists were selected after consideration of the data of Table 1, with the exception of *Pseudomonas* spp. nos. 1 and 2, which were isolated at a relatively late stage from colonies appearing in plates inoculated with a soil suspension and

incubated at 5° C. Table 2 summarizes results obtained after equilibrium between *Botrytis* and the antagonists had been obtained.

Table 2. *Competition between inocula of* B. cinerea *and antagonists*

+ = growth of *Botrytis*. − = *Botrytis* growth inhibited. + − = slight growth.

	Antagonist pre-inoculated in days					
	5° C.		15° C.		25° C.	
Antagonist	0	4	0	2	0	1
Penicillium clavariaeforme, *Pseudomonas* spp. nos. 1 and 2, *Fusarium* spp. nos. 1 and 2. Soil suspension	−	−	−	−	−	−
Streptomyces sp. no. 1, *S. lavendulae*	+	+	−	−	−	−
Trichothecium roseum	+	+ −	+	−	−	−
Cephalosporium acremonium	+ −	+ −	−	−	−	−
Bacillus subtilis	+	+	+ −	−	−	−
Trichoderma viride	+	+	+	−	−	−
Control	+	+	+	+	+	+

At 25° C. all organisms completely controlled the growth and spread of *Botrytis* even when simultaneously inoculated. With few exceptions similar control was obtained at 15° C. But at 5° C. the actinomycetes and bacteria other than *Pseudomonas* spp. were inactive. At this temperature *Penicillium clavariaeforme*, *Pseudomonas* spp. nos. 1 and 2 and *Fusarium* spp. nos. 1 and 2 controlled *Botrytis* with simultaneous inoculation. Use of the soil suspension led to profuse development of bacteria and phycomycetous fungi at all temperatures with complete suppression of *Botrytis*.

It is apparent that while little difficulty was experienced in obtaining antagonists active at relatively high temperatures, the range of organisms active at 5° C. was very limited. The number of organisms from which a selection could be made for use in frames or seed-beds was therefore small.

In spite of these limitations, a number of forms were available which were highly antagonistic over the temperature range 5–25° C. under nutrient conditions approximating to those at the surface of a damaged lettuce leaf. These and others were now tested in the control of disease of lettuce leaves and petioles.

Control of rot of surface-sterilized living leaves

In these experiments the general procedure was to kill small areas of leaf tissue, then to inoculate with *Botrytis* spores either simultaneously with antagonists or 2–4 days later depending on the temperature. After preliminary trials in conjunction with Dr F. J. Newhook, the following method was adopted. Leaves with extensive flat surfaces were washed in tap water and surface sterilized by immersion for 10–15 sec. in 0·1 % mercuric chloride in 5 % alcohol. This solution wetted the leaves immediately. They were then washed in two changes each of sterile tap and distilled water and dried between layers of sterile blotting paper.

The bases of sterile Petri dishes were lined with sterile moist blotting paper and the leaves placed inside. A ring of melted sterile Vaseline approximately 6 mm. internal and 12 mm. external diameter was painted centrally on each half of the lamina. Four such rings were used on large leaves. A fragment of solid carbon dioxide was placed in the centre of the ring for 2–3 sec. and then removed. This produced a localized area of dead tissue 5 mm. in diameter simulating frost damage.

Suspensions of antagonists and *Botrytis* spores in sterile lettuce extract were pipetted separately or in mixtures on to the killed areas. Sufficient was added to cover the area with a thin film. Leaves inoculated in this way could be held at any angle, as there was little danger of spread of the inoculum owing to the high surface tension at the Vaseline surface. After inoculation, the dishes were incubated at temperatures and for periods which varied with the experiment. In general, each treatment included twenty inoculations.

The first of these experiments was carried out at 20° C. One series was inoculated from mixtures of equal volumes of suspensions of antagonists and *Botrytis* spores. In the other, the antagonists were added 2 days before *Botrytis*. The first observations were made 4 days after addition of the pathogen, when the rotting caused by it could be classified as follows.

−	No dead tissue beyond original killed area.
+	Rot extending 1–2 mm. beyond outer edge of ring.
++	Rot extending 5 mm. beyond outer edge of ring.
+++	Rot extending to edge and mid-rib of leaf.

A figure approximating to the total rot in each treatment was obtained by totalling the products (Number of lesions) × (Number of + in each class of rot). For convenience these are expressed as a percentage of the control. On this basis the results are shown in Table 3.

TABLE 3. *Control of rot of detached lettuce leaves by antagonists at* 20° C.

Antagonist	Amount of rot: Simultaneous inoculation	Amount of rot: 2 days pre-inoculation
Control	100 (60)*	100 (60)*
Bacillus brevis	95	3
B. dendroides	10	3
Pseudomonas fluorescens	100	3
Streptomyces lavendulae	100	0
S. violaceus	100	23
Streptomyces sp. no. 1	68	8
Penicillium clavariaeforme	10	10
Trichoderma viride	10	20
Soil suspension	29	14

* 60—actual rating for rot in the control series.

These data show that *Bacillus dendroides*, *Penicillium clavariaeforme* and *Trichoderma viride* gave almost complete control even when simultaneously inoculated.

A large measure of control resulted from addition of a soil suspension. Little or no reduction of rot was given by the others. When inoculated 2 days in advance all treatments behaved similarly in almost completely eliminating rotting.

With further incubation for 3 days these clear-cut results became less well defined. Generally, in the simultaneously inoculated series there was now much less difference between the control and treatments in which substantial reduction of rot had occurred at an earlier stage. In the pre-inoculated series similar effects were observed, but four of the organisms markedly reduced rot and completely eliminated sporulation. Soil suspensions, while not as effective at this stage, still inhibited sporulation.

Similar experiments were carried out at 15° and 5° C. In the former, the pre-inoculated series were incubated for 3 days before addition of *Botrytis* spores. In the latter, leaves of the series pre-inoculated 7 days underwent general physiological breakdown presumably caused by prolonged darkness in the refrigerator.

The results of these experiments are shown in Table 4.

TABLE 4. *Control of rot of detached lettuce leaves by antagonists at* 5° *and* 15° C.

		Amount of rot	
	Antagonist	Simultaneous inoculation	3 days pre-inoculation
15° C.	Control	100 (32)	100 (24)
	Bacillus dendroides	62	42
	Pseudomonas fluorescens	53	38
	Streptomyces lavendulae	56	50
	Streptomyces sp. no. 1	40	38
	Penicillium clavariaeforme	40	33
	Trichoderma viride	31	21
	Soil suspension	22	25
5° C.	Control	100 (56)	—
	Penicillium clavariaeforme	50	—
	Fusarium sp. no. 1	21	—
	Soil suspension	54	—

At 15° C. substantial control of rot was given by all treatments with simultaneous inoculation. Greater control followed pre-inoculation, but the differences were not as striking as at 20° C. As before with further incubation the differences became less pronounced.

At 5° C., when examined 8 days after inoculation, all treatments significantly reduced rot. With further incubation while control by *Penicillium clavariaeforme* remained significant, the others became ineffective. In all, however, sporulation, profuse in the control, was completely inhibited. This was also a general feature at higher temperatures.

Other antagonists were not tested at 5° C. since pure culture work had indicated that little antagonism would have been shown.

Control of rot of unsterile leaves

The work recorded above had in general confirmed expectations based on pure culture studies. While the method of 'sterilization' made it unlikely that the leaf surfaces were in fact completely sterile, nevertheless the conditions were far removed from what might be expected to obtain naturally. In the following, no sterilization was attempted. The leaves were washed in tap water and allowed to dry. Otherwise the procedure was as before. Simultaneous inoculation only was used and the experiment carried out at 15° C. Almost complete control was given by *Bacillus dendroides*, *Pseudomonas* sp. no. 1, *Streptomyces* sp. no. 1, *Penicillium clavariaeforme*, *Trichoderma viride* and *Fusarium* sp. no. 1. *Streptomyces lavendulae* gave much less control under these conditions.

Control of growth of Botrytis *through colonized tissue*

In the above, control of rot presumably depended on inhibition of spore germination or retardation of subsequent growth in mixed culture. The ability of young hyphae of *Botrytis* to penetrate and grow through an area of leaf tissue already colonized by antagonists was now investigated.

Leaves were surface sterilized in the usual way and two concentric rings of Vaseline painted on the upper surface. The areas enclosed by the rings were killed with solid carbon dioxide.

The inner area was inoculated with a *Botrytis* spore suspension; the area between the rings from suspensions of *Penicillium clavariaeforme* or *Pseudomonas* sp. no. 1.

Ten preparations were made in each case. After 5 days' incubation at 20° C. the control series showed typical wet rots extending 5–10 mm. beyond the edge of the outer ring. In the others, the antagonists had developed freely in the intermediate zone. There was some development of *Botrytis* mycelium in the central zone but only slight discoloration beyond the area originally killed. Two days later, leaves in the control series were almost completely rotted. In the other two series the leaf tissue was discoloured in a zone 2–5 mm. beyond the outer ring with *Botrytis* mycelium limited to the central zone. The same effects were produced with *Bacillus dendroides*, *Streptomyces* sp. no. 1 and *Trichoderma viride*.

In a similar experiment, well-washed petioles still attached to the plant were used. The petioles were ringed with Vaseline. The apex was inoculated with a *Botrytis* spore suspension, a zone between rings some 10 mm. away was inoculated with spore suspensions of *Penicillium clavariaeforme* or *Pseudomonas* sp. no. 1. Twelve days after inoculation all petioles of the control series had completely rotted. In the other treatments only half of the petioles had rotted in each case.

Rather different and somewhat unexpected results were obtained with unsterile soil in place of the antagonists. The technique used is illustrated in Fig. 1. The soil was held in place by porcelain cylinders (as used in penicillin assay) which were fixed to the petiole with Vaseline. In each treatment four plants each with four

petioles were used. Observations recorded after 12 days at 15–25° C. are shown in Table 5.

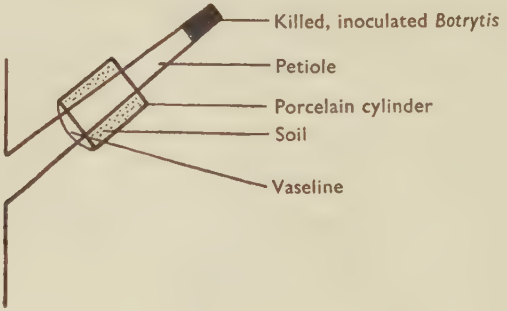


Fig. 1.

Table 5. *Effect of soil on rotting of petiole*

Treatment	Petioles rotted	Petioles healthy
Apex inoculated, soil in cylinder	16	0
Apex inoculated, no soil in cylinder	15	1
Uninoculated, soil in cylinder	1	15
Uninoculated, no soil in cylinder	1	15

It was apparent that soil around the petiole did not prevent passage of *Botrytis* hyphae. In contrast to the previous experiment where profuse development of antagonists occurred in the film of lettuce extract added to the petiole, the relatively small amounts of nutrient available in this experiment probably resulted in only slight stimulation of the growth of the soil microflora. Antibiotic substances, if produced, were insufficient to inhibit the growth of *Botrytis* after diffusion through the petiole as had presumably occurred in the previous experiment.

The inhibition of tissue rot under frame conditions

A further approach to conditions obtaining naturally was now made. In the first experiment seedlings from winter seed-beds were transplanted and allowed to establish themselves for 3 weeks. All dead tissue was then removed and the apices of new leaves ringed with Vaseline in the manner already described. After inoculating the lesions with *Botrytis* simultaneously or 2 days after inoculation with antagonists the plants were placed in a moist chamber at 15–25° C. for a fortnight. By this time, in many cases, rot had spread from the leaf apex to kill a large area of the leaf. In others there was little or no rot extending from the original killed area. Approximately twenty leaves were used for each treatment. Table 6 summarizes the relevant information.

The plants had now become etiolated and were not kept for further observation. The results obtained confirm those above, viz. that pre-inoculation with antagonists may completely control subsequent establishment of *Botrytis*.

TABLE 6. *Control of rot of lettuce leaves by antagonists under frame conditions*

Antagonist	Simultaneous inoculation		3 days pre-inoculation	
	Little or no rot (%)	Extensive rot (%)	Little or no rot (%)	Extensive rot (%)
Control	27	73	17	83
Bacillus dendroides	41	59	75	25
Pseudomonas sp. no. 1	75	25	90	10
Streptomyces lavendulae	20	80	60	40
Streptomyces sp. no. 1	71	29	85	15
Penicillium clavariaeforme	80	20	95	5
Trichoderma viride	50	50	75	25
Fusarium sp. no. 1	95	5	100	0

In the last two experiments to be reported, ordinary culture conditions were maintained. In the first, young potted plants which were healthy apart from some partially decayed leaves now dried and brittle were divided into three groups which were placed in separate compartments of a moist chamber kept in the shade. These were sprayed with (*a*) 1 % glucose solution, (*b*) suspension of *Penicillium clavariaeforme*, (*c*) suspension of *Pseudomonas* sp. no. 1, both in 1·0 % glucose solution. The plants were methodically sprayed to give complete coverage and treatments repeated 8 and 14 days later. For each treatment 100 c.c. of solution were used. A single ordinary slope culture of the antagonists was sufficient for this quantity of liquid.

The temperature of the chamber varied between 5° and 20° C. during the experiment, and approximately forty plants were included in each treatment. The condition of the plants 8 days from the start of the experiment is shown in Table 7, the second column of which gives the number of plants with leaves bearing conidiophores of *Botrytis*.

TABLE 7. *Control of disease of lettuce plants by antagonists under frame conditions*

Treatment	With *Botrytis* conidiophores (%)	Killed (%)
1 % glucose sol.	55	10
1 % glucose sol. + *Penicillium clavariaeforme*	11	3
1 % glucose sol. + *Pseudomonas* sp. no. 1	21	3

A further difference was the much greater necrotic spotting of the control plants especially towards the leaf bases than in those sprayed with antagonists. As the experiment continued, water-soaked patches appeared on the latter and gave rise to sporing areas of *Botrytis*. This sporulation was always much less than in the control. The final position after 25 days is shown below in Table 8.

TABLE 8. *Control of disease of lettuce plants by antagonists under frame conditions*

Treatment	Alive (%)	Killed (%)
1 % glucose sol.	32	68
1 % glucose sol. + *Penicillium clavariaeforme*	87	13
1 % glucose sol. + *Pseudomonas* sp. no. 1	76	24

Spraying with the antagonists thus markedly reduced the mortality. This experiment relied on natural infection by *Botrytis*.

The second experiment carried out somewhat later in the season (May–June) with eighteen plants per treatment was essentially similar. Treatments were applied as before using double the volume of suspension and repeating treatments after 7, 14 and 21 days. As there was little evidence of *Botrytis* on any plant after 9 days all were sprayed with a suspension of spores in distilled water. The progress of the experiment is recorded in Table 9.

Table 9. *Control of disease of lettuce plants by antagonists under frame conditions*

Treatment	Plants dead after			Plants with *Botrytis* conidiophores after		
	14 days	21 days	28 days	14 days	21 days	28 days
1 % glucose sol.	11 %	44 %	78 %	94 %	100 %	100 %
Pseudomonas sp. no. 1	0	11	33	11	39	50
Streptomyces lavendulae	6	17	72	67	100	100
Streptomyces sp. no. 1	6	17	61	44	83	100
Penicillium clavariaeforme	0	6	22	6	22	39
Fusarium sp. no. 1	0	11	33	17	28	39

Thus substantial control of *Botrytis* attack was given by *Pseudomonas* sp. no. 1, *Penicillium clavariaeforme* and *Fusarium* sp. no. 1. In the earlier stages, some improvement was given by *Streptomyces* sp. no. 1 but at no stage did *Streptomyces lavendulae* produce any pronounced effect.

Discussion

The biological control of disease caused by *Botrytis cinerea* envisages the colonization of dead or moribund tissue by saprophytes so as to preclude subsequent invasion by the pathogen. A possible method of doing this was by direct inoculation with selected antagonists and control of disease under frame conditions has been obtained by spraying plants with suspensions of antagonists in 1·0 % glucose solution. The antagonists were selected on the basis of their pronounced antagonism in pure culture and ability to control rot of lettuce leaves under controlled conditions when inoculated simultaneously with the pathogen. The frame experiments were carried out at relatively high temperatures (up to 20° C.) and similar control at lower temperatures has still to be demonstrated. However, the activity shown by the same organisms in the laboratory at 5° C. may be noted.

Frame conditions might be considered favourable to the establishment of selected antagonists, since high humidities may be maintained for long periods following inoculation. Moreover, in the absence of rain splash and except for tissue in contact with the soil, there would probably be little competition from soil saprophytes. Once established, the antagonist might well perpetuate itself under these conditions. Otherwise, repeated application of the antagonist to dead or moribund tissue as produced

would be necessary. In this connexion it is important to emphasize that once established, *Botrytis cinerea* by its rapid invasion of healthy tissue, has a marked competitive advantage over saprophytes limited to dead tissue.

Establishment of antagonists in seed beds would probably be more difficult. Temperatures would generally be lower than in frames and growth would depend very much on climatic conditions following inoculation. Rain would wash spores into the soil, while a dry spell following application of the antagonist might prevent germination or growth of spores already germinated. Competition from soil saprophytes spread by rain splash would be more intense than in frames, since their growth would generally be favoured by conditions suitable for establishment of the antagonist. Indeed, it may well be if conditions *are* suitable for application of the antagonist, inoculation would be unnecessary, as colonization by naturally occurring saprophytes would have obtained to an extent sufficient to preclude *Botrytis*.

Any application of antagonists is likely to have at the most a temporary effect and, since moribund and dead tissue appears throughout the winter, repeated applications would be necessary. In contrast, fungicides having a more persistent effect require fewer applications.

Newhook (1951 *a*, *b*) has in fact shown that repeated application of three antagonists effective under laboratory conditions did not materially decrease incidence of disease in winter seed-beds. He has, however, produced evidence that saprophytic activity on dead lettuce tissue is responsible for a large measure of disease control under natural conditions, confirming earlier observations (Brown & Montgomery, 1948) that survival was higher when plants were grown in hollows and also shown that in one season earthing up seed-beds reduced mortality during the winter. It seems probable that the development of cultural methods encouraging continual and rapid colonization of dead tissue by saprophytes and the stimulation of their growth by addition of suitable materials would be more practical and successful than the direct application of selected antagonists.

REFERENCES

Alexopoulos, C. J. & Herrick, J. A. (1942). Studies in antibiosis between fungi and bacteria, III. *Bull. Torrey bot. Cl.* **69**, 257.

Asthana, R. P. (1936). Antagonism in fungi as a measure of control in 'Red-leg' disease of lettuce. *Proc. Indian Acad. Sci.* **4**, 201.

Bamberg, R. H. (1931). Bacteria antibiotic to *Ustilago zeae*. *Phytopathology*, **21**, 881.

Brian, P. W. & Hemming, H. G. (1945). Gliotoxin, a fungistatic metabolic product of *Trichoderma viride*. *Ann. appl. Biol.* **32**, 214.

Brown, W. & Montgomery, N. (1948). Problems in the cultivation of winter lettuce. *Ann. appl. Biol.* **35**, 161.

Fawcett, H. S. (1912). Rep. Plant Pathologist. *Fla. Agric. Exp. Sta. Ann. Rep.*

Hendrickx, F. L. (1937). Sur l'antagonisme existant entre une bactérie et l'agent de la maladie de l'Orme. *C.R. Soc. Biol., Paris*, **126**, 99.

Khudiakoff, J. P. (1935). The lytic action of soil bacteria on parasitic fungi. *Microbiol. U.S.S.R.* **4**, 193.

Koch, L. W. (1934). Investigations on black-knot of plums and cherries. II. *Sci. Agric.* **15**, 80.

Matsumoto, T. (1939). Need of reinvestigation on the use of *Trichoderma* as a means of biological control. *J. Soc. Trop. Agric., Taiwan*, **11**, 322.

Newhook, F. J. (1951*a*). Microbiological control of *Botrytis cinerea* Pers. 1. The role of pH changes and bacterial antagonism. *Ann. appl. Biol.* **38**, 169.

Newhook, F. J. (1951*b*). Microbiological control of *Botrytis cinerea* Pers. II. Antagonism by Fungi, Actinomycetes and micro-organisms occurring in soil suspensions. *Ann. appl. Biol.* **38**, 185.

Porter, C. L. (1932). Mixed cultures of bacteria and fungi. *Proc. Ind. Acad. Sci.* **41**, 149.

Savastano, G. & Fawcett, H. S. (1929). A study of decay in Citrus fruits produced by inoculations with known mixtures of fungi at different constant temperatures. *J. Agri. Res.* **39**, 163.

Savastano, G. & Fawcett, H. S. (1930). *Estratti dagli Annali della R. Stazione Sperimentale di Agrumicoltura e Frutticoltura in Acireale*, **11**, 1.

Vasudeva, R. S. (1930). Studies in the physiology of parasitism. XII. *Ann. Bot., Lond.*, **44**, 557.

(*Received* 16 *February* 1950)

THE CONTROL OF DISEASES OF LETTUCE BY THE USE OF ANTAGONISTIC ORGANISMS

II. THE CONTROL OF *RHIZOCTONIA SOLANI* KÜHN.

By R. K. S. WOOD

Imperial College of Science and Technology, London, S.W.7

The antagonism of a number of bacteria, actinomycetes and fungi in pure culture on soil extract agar has been investigated. A considerable number of each of the three groups were highly antagonistic at 25° C., fewer were active at 15° C. and only a very limited number were effective at 5° C.

In greenhouse experiments damping-off of lettuce seedlings was substantially controlled by selected antagonists in sterile sand, soil or in sand/soil mixtures. Under similar conditions in unsterile soil, forms highly antagonistic in pure culture were generally ineffective in controlling disease. In one experiment control of disease in natural soil was obtained by the use of soil amendments, the effect being enhanced by the inoculation of one of these amendments with suspensions of antagonists.

Attempts to control disease in the field by the use of soil amendments are described. In one year a substantial increase in the stand of healthy seedlings was obtained with certain treatments.

In an earlier paper (Wood, 1951) reference was made to the relative importance of *Botrytis cinerea* and *Rhizoctonia solani* in reducing the stands of lettuce seedlings in winter seed-beds and frames. An account was given of the control of disease caused by *Botrytis cinerea* by the direct use of antagonistic organisms. Similar investigations in the control of the *Rhizoctonia* phase of the attack were begun in 1947 and are reported below.

Antagonism to *Rhizoctonia solani* in pure culture has been reported by a number of workers. Weindling (1932, 1934*a*) showed that the hyphae of this pathogen were actively parasitized by *Trichoderma viride* which produced a highly toxic substance in liquid culture. A number of other fungi, including *Botrytis cinerea*, acted similarly (Weindling 1934*b*). This work was confirmed by Allen & Haenseler (1935). A strain of *Bacillus simplex* isolated by Cordon & Haenseler (1939) produced in a potato/dextrose/peptone medium a thermostable substance of high toxicity. Sanford (1946) demonstrated that a number of bacteria antagonistic to *Actinomyces scabies* similarly affected *Rhizoctonia solani*.

Much work on the biological control of disease caused by *R. solani* followed Weindling's discovery of the active parasitism of this pathogen by other fungi. Thus Weindling & Fawcett (1936) demonstrated control of attack of citrus seedlings in peat by the addition of a spore suspension of *Trichoderma lignorum* and control of disease in unsterile soil by acidification to pH 4·0. This work was confirmed by Allen & Haenseler (1935) who obtained a significantly increased stand of cucumber

and pea seedlings in infected unsterile soil by incorporating cultures of *T. lignorum*. Cordon & Haenseler (1939) showed that reduction of disease followed the addition to unsterile soil of suspensions of a strain of *Bacillus simplex* known to be antagonistic in pure culture. Jaarsveld (1942) using sterile soil or sand substantially controlled attack of cabbage seedlings with a number of fungi. Using different techniques Blair (1943) demonstrated that in unsterile soil, the growth rate of the mycelium was retarded by various organic amendments. Similar retardation was obtained in sterile soil by adding cultures of various soil fungi. The effect of soil amendments in controlling attack of potato sprouts in the field was studied by Sanford (1946, 1947) who found that only cornmeal and nitrogen salts caused consistent reduction of disease.

Similar work has been done with a number of other pathogens and in particular *Ophiobolus graminis*, *Helminthosporium sativum* and *Fusarium* spp., causing various cereal diseases, *Phymatotrichum omnivorum* causing cotton root rot and species of *Pythium* and *Phytophthora* causing damping-off of a number of plants. The possibility of using antagonists or soil amendments to control these diseases on a field scale has been considered in these studies but only with cotton root-rot has substantial success been reported.

General experimental methods

A study was first made of the antagonism of a wide range of organisms in pure culture under poor nutrient conditions. Selected organisms were then tested for their ability to control damping-off of lettuce seedlings at first under conditions of complete or 'partial' sterility and then in unsterile soil. The control of disease in winter seed-beds by addition of antagonists or soil amendment was then attempted.

A single isolate of *Rhizoctonia solani* was used throughout the work. It was obtained from a diseased seedling from a winter seed-bed.

Soil extract agar was used in pure culture studies. This was prepared by adding 500 g. of seed-bed soil to 500 c.c. of tap water and standing for 1 hr. The mixture was passed through a cotton wool pad and cleared by centrifuging for 10 min. at 2500 r.p.m. The supernatant liquid was made up to 1000 c.c. with tap water and 1 g. of glucose and 15 g. of agar added before sterilization at 125° C. for 30 min.

Material for soil inoculation was generally obtained by culturing the pathogen and antagonists on the following: silver sand 95 g., ground maizemeal 5 g., water 30 c.c. 200 g. of this mixture were placed in 500 c.c. conical flasks and sterilized at 135° C. for 30 min.

All organisms grew well on this medium. The antagonists were generally inoculated from suspensions. The actinomycetes and bacteria were distributed by thoroughly shaking the culture 4–5 days after inoculation

Cultures were incubated at 25° C. for 2 weeks. They were then broken up and made uniform by passing three to four times through a 2 mm. sieve. In pathogenicity tests, lettuce seedlings var. Imperial and seed-bed soil were used.

The organisms used were, in general, those isolated during similar work with *Botrytis cinerea*. The following showed the most striking antagonistic effects:

Group I. Bacteria. *Bacillus subtilis*, *B. brevis*, *B. dendroides*, *B. anthracoides*, *B. megatherium*, *Pseudomonas fluorescens*, *Ps. aeruginosa*.

Group II. Actinomycetes. *Streptomyces violaceus*, *S. albus*, *S. antibioticus*, *S. lavendulae*, two unidentified *Streptomyces* spp. and Meredith's actinomycete.

Group III. Penicillia. *Penicillium clavariaeforme*, *P. patulum* and other unidentified spp.

Group IV. Fungi imperfecti. *Trichoderma viride* and *Fusarium* spp.

After inoculation, soil was placed in glass jars 2·5 cm. deep and 6·5 cm. internal diameter, with rust-proof lids which were placed loosely on during incubation and in the interval between sowing of seeds and emergence of seedlings. These jars held approximately 100 g. of soil or sand. While the lids were in place, water loss was negligible and since the work was concerned only with young seedlings the lack of depth of soil was immaterial.

The soil was adjusted to 40–50 % of its water-holding capacity before inoculation. No attempt was made to maintain it at constant level during the period of the experiment. Water loss was negligible until the lids were removed on emergence of the seedlings.

Seeds were always sown as follows: The soil was added to the jar and levelled. A metal disk in which were punched equidistantly thirty-two holes 4 mm. in diameter was placed on the surface and the soil lightly compacted. A single seed was placed in each hole and pressed 5 mm. into the soil. The disk was then removed and the surface again levelled. No water was added at this stage. After sowing, the jars were placed in a greenhouse the temperature of which varied between 15 and 20° C. Treatments were replicated four or fivefold. After emergence the seedlings were examined at 3–4-day intervals when dead plants were removed and counted.

Antagonism on agar media

The procedure resembled that already described for *Botrytis cinerea* (Wood, 1951). It differed in that soil extract agar was used and that the inoculum of the pathogen was a 4 mm. disk taken from a young culture on the same medium.

The relative behaviour of antagonists and the pathogen was also similar and the best pictures of antagonistic properties is again given by the suppression of radial growth.

In Table 1 a radial growth of > 29 mm. is taken to indicate little or no antagonism; 25–29 mm. slight antagonism; 15–24 mm. pronounced antagonism and < 15 mm. very pronounced antagonism. In certain instances bacterial antagonists spread rapidly over the surface of the agar completely inhibiting the growth of *Rhizoctonia*.

This is recorded as 'envelopment'. The results for each of the four groups of antagonists previously listed are shown together.

Table 1. *Tests of antagonism on soil extract agar*

Degree of antagonism	Radial growth of *R. solani* (mm.)	Group I (bacteria)			Group II (actinomycetes)			Group III (penicillia)			Group IV (*Trichoderma viride* and *Fusarium* spp.)	
		5°	15°	25°	5°	15°	25°	5°	15°	25°	5°	15° (C.)
None	>29	30	17	11	12	1	6	17	9	4	11	3
Slight	25–29	—	—	2	1	4	1	1	1	1	0	1
Pronounced	15–24	—	11	8	—	6	5	2	3	4	3	1
Very pronounced	<15	—	—	2	—	1	2	—	1	1	1	9
Doubtful	Enveloped	—	—	5	—	—	—	—	—	—	—	—
Total treated		30	28	28	13	12	14	20	14	10	15	14

The following are the general conclusions. Only a few of the bacteria, actinomycetes and penicillia showed antagonism at 5° C. There was a marked increase in activity at 15° C. but little or no further increase at 25° C. In this respect *Rhizoctonia* behaved differently from *Botrytis*. At 25° C. antagonism to *Rhizoctonia* was less frequently observed than to *Botrytis*. As before, a limited number of penicillia showed striking antagonism at all temperatures. In almost all cases antagonism took the form of 'zones' free of *Rhizoctonia* hyphae.

Increased activity at 15° C. was also exhibited by *Trichoderma viride* and the *Fusarium* spp. They were not tested at 25° C. As with *Botrytis*, 'zones' free of mycelium were absent in almost every case.

In all groups, antagonists showing marked activity towards *Botrytis*, generally behaved similarly towards *Rhizoctonia* but on the whole there was less antagonism to the latter. This may have been caused by the method of selection in the first place or the difference in the nutrient levels of the media which were used.

In the second series of experiments a 15 mm. square in plates of soil extract agar was inoculated with various antagonists and further inoculated at the centre with 4 mm. disks taken from a young culture of *Rhizoctonia*. The pathogen was inoculated simultaneously with antagonists or after the latter had been incubated for 4, 2 or 1 days at 5°, 15° and 25° C. respectively. As with *Botrytis*, in the majority the growth of the pathogen was either almost completely inhibited (−) or little affected by the antagonist (+). Intermediate effects were occasionally observed (+ −) where the hyphae grew irregularly from the inoculum, in some for a very limited distance, in others as far as the edge of the antagonist colony to grow away and spread over the agar surface. The reason for the anomalous behaviour of such hyphae is not known. It may have been caused by variability of individual hyphae or to unequal development of the antagonist on the inoculated area. The results are summarized in Table 2.

Penicillium clavariaeforme, *Pseudomonas* spp. nos. 1 and 2 and the soil suspension

TABLE 2. *Competition between inocula of* R. solani *and antagonists*

+ = Growth of *R. solani*. − = *R. solani* growth inhibited. +− = slight check.

	Antagonist pre-inoculated in days					
	5° C.		15° C.		25° C.	
Antagonist	0	4	0	2	0	1
Penicillium clavariaeforme, Pseudomonas spp. nos. 1 and 2, soil suspension	−	−	−	−	−	−
Bacillus dendroides	Not tested		+	−	+−	−
Streptomyces sp. no. 1	Not tested		−	−	−	−
S. lavendulae	Not tested		−	−	−	−
Trichoderma viride	Not tested		+	+	−	−
Bacillus subtilis	Not tested		+	+	+−	−
Control	+	+	+	+	+	+

controlled the growth of *Rhizoctonia solani* at all temperatures. Similar control was given by the others when pre-inoculated for 1 day at 25° C.

It was apparent from the work recorded above and from other similar experiments that while a considerable number of organisms were antagonistic at temperatures of 15° C. and higher, the number showing activity at 5° C. was very limited. The general inactivity at 5° C. had not been determined when the first selection of antagonists was made for experiments on disease control. Much of this work was carried out at 15° C. or higher—at these temperatures antagonism in pure culture was pronounced. Later, however, it was apparent that many of the forms used would have grown little at 5° C. and this in itself would have precluded their use as possible agents of control under frame or field conditions. However, one form used extensively, *Penicillium clavariaeforme*, was highly antagonistic at all temperatures.

Control of disease under greenhouse conditions

The general plan of these experiments was to inoculate the soil medium with the pathogen and the antagonist, to incubate for 2–3 weeks and then to sow with lettuce seed and make counts of emergence and damping-off. In earlier experiments, 'partially' or completely sterilized sand or sand/soil mixtures were used. In later experiments unsterile soil was used. Great care was always taken to thoroughly incorporate the inocula with the soil by hand-mixing and passing repeatedly through a 2 mm. sieve. In spite of this, in some experiments the individual replicates of series behaved very differently. Similar lack of uniformity in experiments with this pathogen have been reported by Sanford (1947).

Sterile conditions. In one experiment, 10 c.c. of sterile soil extract solution was added to 100 g. of sterile silver sand in jars. Five 4 mm. disks from a young culture of the pathogen on soil extract agar were placed equidistantly 5 mm. below the surface. 10 c.c. of suspensions of the antagonists in sterile soil extract were added, the jars incubated for 3 weeks at 20° C. and then sown. The summary of results recorded 3 weeks later is given in Table 3.

Table 3. *Control of damping-off in sterile sand with suspensions of antagonists*

Treatment	Seedlings (%) Non-emergent	Damped-off	Final stand
Rhizoctonia solani	23	49	28
R. solani + *Streptomyces* sp. no. 1	7	0	93
R. solani + *S. lavendulae*	17	31	52
R. solani + *Penicillium clavariaeforme*	14	5	81
R. solani + *Trichoderma viride*	11	10	79
Significant difference $P=0.05$	—	18	22
$P=0.01$	—	24	30

There was considerable variation between the replicates of the control and *Streptomyces lavendulae* series. Thus the final stands were 3, 21, 18, 66 and 78, 57, 24, 52 respectively and the differences of means between the two were significant only at the $P=0.05$ level. The addition of the other antagonists gave highly significant decrease of post-emergence damping-off and increase of stand. The seedlings in the jars treated with *Penicillium clavariaeforme* were suffering phytocidal damage, being stunted with poorly developed root systems.

A different method of inoculation was then used, again with sterile silver sand. The pathogen was added as 10 % by weight of a sand/maizemeal culture. Thereafter the procedure was as above, a somewhat different range of organisms being used. The results are shown in Table 4.

Table 4. *Control of damping-off in sterile sand with sand/maizemeal cultures*

Treatment	Seedlings (%) Non-emergent	Damped-off	Final stand
Rhizoctonia solani	20	33	47
R. solani + *Bacillus subtilis*	5	10	85
R. solani + *Streptomyces* sp. no. 1	18	12	70
R. solani + *S. violaceus*	3	28	69
R. solani + *Penicillium clavariaeforme*	95	5	0
R. solani + *Trichoderma viride*	2	12	86
Significant difference $P=0.05$	10	—	21
$P=0.01$	14	—	29

Again, with one exception, all treatments increased the final stand. In the series treated with *Penicillium clavariaeforme*, however, profuse development of the antagonist had occurred and with few exceptions the seeds were ungerminated. No germination occurred after washing and placing in moist chambers. It was apparent that the phytocidal damage already noted had here developed to cause almost complete failure of germination.

A further experiment resembled that first described. It differed in that silver sand

was replaced by a mixture of 3 parts soil: 1 part silver sand. The results are shown in Table 5.

TABLE 5. *Control of damping-off in sterile soil with suspensions of antagonists*

Treatment	Seedlings (%)		
	Non-emergent	Damped-off	Final stand
Rhizoctonia solani	80	14	6
R. solani + *Bacillus subtilis*	2	39	59
R. solani + *B. dendroides*	6	7	87
R. solani + *Streptomyces* sp. no. 1	3	7	90
R. solani + *S. lavendulae*	2	17	81
R. solani + *Penicillium clavariaeforme*	6	8	86
R. solani + *Trichoderma viride*	2	0	98
Significant difference $P=0{\cdot}05$	—	17	20
$P=0{\cdot}01$	—	23	26

Here the addition of antagonists in all cases almost completely controlled pre-emergence damping-off, which, as in the next experiment, was largely responsible for the low final stand in the control.

In the *Bacillus subtilis* series and to a lesser extent in the *Streptomyces lavendulae* series post-emergence damping-off did occur. It was unimportant in the other series. All treatments significantly increased final stands, and in this case there was no indication of phytocidal damage in the *Penicillium clavariaeforme* series.

In the final experiment of this series to be reported, sand/maizemeal cultures of both pathogen and antagonists were used to inoculate a steam sterilized mixture of 3 parts soil: 1 part silver sand at 5 % and 10 % by weight respectively. In the controls, uninoculated cultures replaced those of antagonists and in addition a further series was included where the pathogen inoculum was similarly replaced. The results are recorded in Table 6.

TABLE 6. *Control of damping-off in sterile soil with sand/maizemeal cultures*

Treatment	Seedlings (%)		
	Non-emergent	Damped-off	Final stand
Uninoculated media	11	8	81
Rhizoctonia solani	42	20	38
R. solani + *Bacillus subtilis*	42	6	52
R. solani + *B. brevis*	49	8	43
R. solani + *Streptomyces* sp. no. 1	44	6	50
R. solani + *S. lavendulae*	35	6	59
R. solani + *Penicillium clavariaeforme*	30	4	66
R. solani + *Trichoderma viride*	27	11	62
R. solani + mixture of all	33	5	62
Significant difference $P=0{\cdot}05$	—	10	18
$P=0{\cdot}01$	—	14	24

Increased stands significant at the $P=0{\cdot}01$ level were given by *Penicillium clavariaeforme* and *Trichoderma viride* and at the $P=0{\cdot}05$ level by *Streptomyces lavendulae*. *Streptomyces* sp. no. 1 and *Bacillus subtilis* were less effective and *B. brevis* was ineffective.

These and other experiments made it clear that under conditions where the competitive influences of the normal soil flora were eliminated a number of antagonists were able to control the spread of actively parasitic hyphae of *Rhizoctonia solani* from inocula introduced into soil, sand or mixtures. The temperatures used varied between 15° and 20° C. but it is reasonable to suppose that in the case of *Penicillium clavariaeforme* similar effects would have been produced at lower temperatures.

Unsterile conditions. In an experiment resembling that described immediately above, unsterile soil was used and the inocula of antagonist and pathogen increased to 25 and 10 % by weight respectively. Six days after sowing the stand was significantly increased by the majority of the organisms tested (total of 12). But this effect did not persist. Two weeks later, with the exception of the uninoculated series, only isolated seedlings remained in each treatment.

Experiments with maizemeal inocula using mixtures of antagonists instead of individual species did not result in significant increase in stand notwithstanding the fact that in some treatments *Penicillium clavariaeforme* and *Streptomyces lavendulae* developed freely in the interstices and on the surface of the soil, giving the soil the pronounced smells characteristic of these organisms.

In the above, the antagonists were added in the form of 2–3-week old cultures. In effect the addition was one of a relatively large bulk of the organism together with the by-products of its metabolism. The quantity of unused nutrient material transferred was probably small and it may well have been that in the majority of cases relatively little growth of the antagonist occurred after inoculation of the soil. It was certainly true that only on a few occasions was there any macroscopic evidence of such growth.

In the following experiment, a relatively large bulk of unused food material was sprayed with suspensions of selected antagonists before adding to the soil. In this way it was hoped to establish the antagonists on the material before it was colonized by the soil microflora. In addition, series were included in which dried maizemeal was added to cultures before incorporating into the soil. The treatments are indicated in Table 7. After 2 weeks at 17–20° C. the soil surface in treatments 3, 5, 6, 7, 10, 12, 13, 14 and 17 was covered with a profuse growth of mycelium binding together the upper layers. The soil of treatments 4, 8 and 15 bore numerous coremia of *Penicillium clavariaeforme* which could also be seen in the interstices. Similarly, *Streptomyces lavendulae* grew freely on the surface of the soil in treatments 9 and 16. Sporing pustules of *Trichoderma viride* were not seen.

The following are the conclusions from these results. Addition of lime decreased the final stand largely owing to increased pre-emergence damping-off. Addition of

TABLE 7. *Control of damping-off in unsterile soil with soil amendments and suspensions of antagonists*

Treatment	Seedlings (%)		
	Non-emergent	Damped-off	Final stand
1. Uninoculated	25	28	47
2. *Rhizoctonia solani*	14	59	27
3. *R. solani* + 1 % dried grass	23	4	73
4. *R. solani* + 1 % dried grass + *Penicillium clavariaeforme* spores	16	1	83
5. *R. solani* + 1 % dried grass + *Streptomyces lavendulae* spores	24	6	70
6. *R. solani* + 1 % dried grass + *Trichoderma viride* spores	19	5	76
7. *R. solani* + 1 % maizemeal	47	25	28
8. *R. solani* + 1 % maizemeal + *Penicillium clavariaeforme* spores	28	11	61
9. *R. solani* + 1 % maizemeal + *Streptomyces lavendulae* spores	34	14	52
10. *R. solani* + 1 % maizemeal + *Trichoderma viride* spores	40	29	31
11. *R. solani* + 1 % hydrated lime	69	29	2
12. *R. solani* + 1 % hydrated lime + 1 % dried grass	52	35	13
13. *R. solani* + 1 % hydrated lime + 1 % dried grass + *Streptomyces lavendulae* spores	68	29	3
14. *R. solani* + 1 % maizemeal + 20 % uninoculated culture	18	28	54
15. *R. solani* + 1 % maizemeal + 20 % culture *Penicillium clavariaeforme*	26	19	55
16. *R. solani* + 1 % maizemeal + 20 % culture *Streptomyces lavendulae*	18	32	50
17. *R. solani* + 1 % maizemeal + 20 % culture *Trichoderma viride*	25	25	50
Significant difference $P = 0{\cdot}05$	12	14	12
Significant difference $P = 0{\cdot}01$	16	18	16

dried grass gave substantial control of post-emergence damping-off and increased the final stand. Inoculation of the grass with antagonists gave no further effect. Use of 20 % by weight of an uninoculated sand/maizemeal medium and 1 % dried maizemeal resulted in an increased stand, and again no further effect was obtained by inoculation with antagonists. Addition of 1 % maizemeal alone had no effect on final stand and this result, although not strictly comparable, is incompatible with that of treatment 14. In the series, 7, 8 and 9, inoculation with *Penicillium clavariaeforme* and to a lesser extent with *Streptomyces lavendulae* increased the stand but there was still some loss of seedlings in spite of relatively profuse development of the antagonists.

It was clear that control of disease in unsterile soil was much more uncertain than in sterile soil or sand. The positive results which were obtained could in some instances be attributed to the activity of the antagonists—in others the amendment alone was as effective. Many organisms, highly active in pure culture and in control of disease under sterile conditions, were completely ineffective in natural soil.

Even in the few cases where control was obtained, the antagonists were added to the soil simultaneously with the pathogen, i.e. the control obtained may have been a measure only of their ability to control the spread of the pathogen from the inoculum through the soil. Greenhouse experiments aiming at the control of the pathogen after establishment were unsuccessful largely owing to failure of the inoculum to persist sufficiently long.

Control of disease under field conditions

Two areas, 1 and 2, were available at Slough where the survival of lettuce either planted out in the autumn or in winter seed-beds had generally been very poor.

These areas were divided into four blocks containing a number of plots each 5 × 3 ft. The general procedure was to treat the soil of these plots in various ways during the summer preparatory to sowing as winter seed-beds in September. All treatments were in quadruplicate and randomized within the blocks.

During 1947, sand/maizemeal cultures of *Streptomyces lavendulae*, *Bacillus dendroides*, *Penicillium clavariaeforme* and *Trichoderma viride*, individually or as mixtures, were incorporated in the upper 3 in. of the soil. 1000 g. of the cultures were added to individual plots on each of four occasions during the summer. The fungal treatments were also combined with acidification of the soil to pH 5·0 with sulphuric acid. The effect of adding green manure with and without acidification and of partial sterilization with formalin was also studied. All plots were kept free of weeds during the summer and sown each with 1·0 g. of Sutton's Imperial lettuce seed in four 5 ft. drills on 25 September.

Examination 5 weeks later clearly showed that acidification resulted in uneven stands of badly checked seedlings. With other treatments, good even stands of healthy seedlings were obtained. Periodic examination during the winter revealed no

differences between the treatments and when finally examined on 12 March 1948, only isolated plants remained.

Soil treatments were continued during the summer of 1948. By this time, laboratory work had indicated that the addition of antagonists on a practicable scale was unlikely to reduce disease in the field. Accordingly such treatments were abandoned in favour of others which the experience of workers in this field had shown to result in a material change in the composition and activity of the soil flora during the summer months. The same plots were used: it was assumed that except for those to which acid had been added, the effect of the previous season's treatments would have disappeared. The acid treated plots were used again for similar treatments.

The following additional treatments were applied to individual plots:

Area 1

(1) *Formalin.* 400 c.c. formalin diluted to a 2·0 % solution. (pH 6·6.)
(2) *Green manure.* A total 4000 g. green manure as freshly cut lawn mowings forked into upper 3–4 in. (pH 6·4.)
(3) *Green manure and lime.* As in (2). In addition 250 g. hydrated lime to each plot. (pH 7·2.)
(4) *Dry.* Soil repeatedly forked and ridged in sun to dry out upper layers. Covered with frames until 2 weeks before sowing. Well watered during the latter period. (pH 6·8.)
(5) *Wet.* Soil kept wet by heavy watering. Holes 6 in. deep at intervals of 9 in. were made and 10 gal. of water periodically added to each plot until 2 weeks before sowing. Forked to dry out during the latter period. (pH 6·8.)
(6) *Mustard.* Three crops of white mustard thickly sown, cut and forked into upper layers. (pH 6·8.)
(7) *Control.* Soil undisturbed.

Area 2

(1) *Frame and green manure.* As (2) above but covered with frames and kept moist until a week before sowing. (pH 6·8.)
(2) *Formalin and green manure.* Combination of (1) and (2) above. (pH 6·6.)
(3) *Straw.* 500 g. chopped straw forked into upper 3–4 in. (pH 6·2.)
(4) *Maizemeal.* 250 g. finely ground maizemeal forked into upper 3–4 in. (pH 6·8.)
(5) *Control.* Soil undisturbed. (pH 6·8.)
(6) *Control.* Soil undisturbed. (pH 6·8.)

The plots were kept free of weeds during the summer and a fortnight before sowing were forked over and well stirred with a heavy rake. On 29. ix. 48 three 5 ft. drills were sown in each plot with 0·75 g. of Sutton's Imperial lettuce seed.

When first examined a month later there was an even stand along the drill-length in all plots on Area 1 except in those acidified and in one of the dry series. In the latter two-thirds of the drills contained plants. In the former, emergence was uneven and seedlings were stunted and unhealthy in appearance.

On Area 2 there was an even stand of seedlings on all plots.

A month later only occasional dead plants were found on Area 2, but damping-off patches were appearing on Area 1. Estimates were made of the length of drill affected in this way. Isolations from plants in such patches gave 90 % *Rhizoctonia*

solani cultures. Owing to the extensive phytocidal damage no estimates were made for the acidified plots. The data are shown in Table 8.

Table 8. *Incidence of damping-off patches in treated plots of winter seed-beds*

Treatment	Average percentage of drill as damped-off patches
1. Formalin	2·2
2. Green manure	0
3. Green manure + lime	0
4. Dry	21·1
5. Wet	0·8
6. Mustard	1·1
7. Control	4·4

The plants on Areas 1 and 2 were lifted, washed and examined on 21. i. 49 and 9. ii. 49 respectively. Relatively few were moribund as a result of *Botrytis* attack. A larger proportion showed lesions on hypocotyls and roots.

A summary of the data is given in Table 9.

Table 9. *Effect of soil treatments on stand of seedlings in winter seed-beds*

	Average seedlings per plot (total)	Average seedlings per plot			Average weight per seedling (g.)
		Healthy	Root lesions	Attacked *Botrytis*	
Area 1					
1. Formalin	376	307	57	12	0·52
2. Green manure	407	374	26	7	0·54
3. Green manure + lime	392	333	48	11	0·55
4. Dry	231	154	62	15	0·46
5. Wet	397	354	31	12	0·54
6. Mustard	412	359	41	12	0·55
7. Control	330	250	68	12	0·51
Significant difference $P=0·05$	96	99	20	—	—
$P=0·01$	128	134	27	—	—
Area 2					
1. Frame + green manure	362	325	28	9	0·66
2. Formalin + green manure	342	309	28	5	0·69
3. Straw	325	296	22	7	0·57
4. Maizemeal	359	317	35	7	0·57
5. Control	294	244	40	10	0·48
6. Control	270.	234	27	9	0·61
Significant difference $P=0·05$	72	77	—	—	0·11
$P=0·01$	97	104	—	—	0·16

There was considerable variation between the replicates as shown by the high differences required for statistical significances. This was also a general feature of laboratory experiments finding confirmation in the work of Sanford (1946, 1947). It probably indicates the rather delicate nature of the mechanism of control. Depending as it does on competition within a limited environment, comparatively

slight local variations might well have disproportionate effects in tipping the balance in directions leading to suppression or dominance of the pathogen.

The application of green manure which increased the stand of healthy seedlings in 1948 had been ineffective in 1947. This may have been due to the fact that in the warm dry summer of 1947 a substantial part of the green manure remained undecomposed while in 1948 it had practically disappeared at the time of sowing.

Additional treatment, viz. lime, formalin or covering with a frame, had no further effect. The stand of healthy seedlings was significantly lower in the plots kept dry during the summer. On Area 1 where root lesions were more frequent, treatments giving increased numbers of healthy plants also gave fewer diseased seedlings. There were no significant differences at this stage in the numbers of seedlings moribund as a result of *Botrytis* attack or, on Area 1, in the average seedling weight. On Area 2 the average seedling weight was significantly lower on some plots—the reasons for this were not apparent.

Discussion

Using soil extract agar, a considerable proportion of the species tested were strongly antagonistic at high temperatures. At low temperatures relatively few were active and it is only such forms which could have been considered for use in frames and seed-beds during the winter. It does, however, seem possible that by incubating soil dilution plates at 5° C. and by similar methods the range of low-temperature forms could be extended.

The greenhouse experiments on control of damping-off were limited to the investigation of the effect resulting from inoculation of the pathogen simultaneously with the antagonist or soil amendment. Under sterile conditions, successful control was frequently obtained but with natural soil results were erratic. Experiments to control disease in soil in which the pathogen had been established for some time were not possible owing to failure of the pathogen to persist sufficiently long. It is to be noted, too, that these experiments were carried out at relatively high temperatures (15–20° C.). In view of the behaviour of the majority of the antagonists in pure culture at lower temperatures, it seems probable that even under sterile conditions little success would have been obtained.

Under field conditions, addition of antagonists alone had no effect. Acidification of the soil with sulphuric acid had adverse effects on germination or subsequent growth of the seedlings and was of no value as a control method in these experiments. In 1948 incidence of attack in soil kept dry during the summer was higher than in untreated soil and lower in soil which had been kept wet. This agreed with the general observation at Slough that attack by *Rhizoctonia* is more severe following a dry summer and worse on light than on heavier soil. A possible explanation is that the pathogen becomes wholly or partially dormant in the form of sclerotia in the summer and the destruction of these and also of mycelium is highest under conditions favouring microbiological activity. This might lead to a reduction of foci of infection in the following autumn.

The addition of cultures of antagonists on a practicable scale is unlikely to have a material effect in seed beds and only slight increases in control could be expected in frames even under the most favourable conditions. In both, a more promising line of attack would be the development of practices designed to sustain at a high level the activity of the soil microflora during the summer months, e.g. by watering in dry seasons, frequent digging in wet seasons and the addition of cheap organic amendments.

REFERENCES

Allen, M. C. & Haenseler, C. M. (1935). The antagonistic action of *Trichoderma* on *Rhizoctonia* and other soil fungi. *Phytopathology*, **25**, 244.

Blair, I. D. (1943). Behaviour of the fungus *Rhizoctonia solani* Kühn in the soil. *Ann. appl. Biol.* **30**, 118.

Cordon, T. C. & Haenseler, C. M. (1939). A bacterium antagonistic to *Rhizoctonia solani*. *Soil Sci.* **47**, 207.

Jaarsveld, A. (1942). Der Einfluss verschiedener Bodenpilze auf die Virulenz von *Rhizoctonia solani* Kühn. *Phytopath. Z.* **14**, 1.

Sanford, G. B. (1946). Soil borne diseases in relation to the microflora associated with various crops and soil amendments. *Soil Sci.* **61**, 9.

Sanford, G. B. (1947). Effect of various soil supplements on the virulence and persistence of *Rhizoctonia solani*. *Sci. Agric.* **27**, 533.

Weindling, R. (1932). *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology*, **22**, 837.

Weindling, R. (1934*a*). Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopathology*, **24**, 1153.

Weindling, R. (1934*b*). Various fungi found to be parasitic on *Rhizoctonia solani*. *Phytopathology*, **24**, 1141.

Weindling, R. & Fawcett, H. S. (1936). Experiments in the control of *Rhizoctonia* damping-off of Citrus seedlings. *Hilgardia*, **10**, 1.

Wood, R. K. S. (1951). The control of diseases of lettuce by the use of antagonistic organisms. I. The control of *Botrytis cinerea* Pers. *Ann. appl. Biol.* **38**, 203.

(*Received* 16 *February* 1950)

BACTERIAL WET ROT OF POTATO TUBERS FOLLOWING *PHYTOPHTHORA INFESTANS*

By W. J. DOWSON and D. RUDD JONES
Botany School, Cambridge

Potato tubers infected with *Phytophthora infestans* in the field produce abnormal amounts of liquid which often appear on the surface of tubers kept in a saturated atmosphere. Under these conditions a soft rot, associated with bacteria, develops.

Healthy tubers artificially infected with pure cultures of *P. infestans* produce a similar liquid and, if further inoculated with pure cultures of certain bacteria, develop a wet rot which spreads if the bacteria are pathogenic or is confined to the zone of fungal invasion if the bacteria are saprophytes.

Sap extracted from *Phytophthora*-infected tissue contains more sugar and has a greater osmotic pressure than sap extracted from healthy tissue. Thus, water may be withdrawn from healthy tissue which would result in the infected tissue containing more liquid than does the healthy tissue.

It has long been known that stored potatoes may exhibit two chief types of rot, one dry, the other wet. Thus Delacroix (1903) distinguished a dry rot due to fungi, and a wet rot in which he found a preponderance of various bacteria. Delacroix also noted intermediate types of rot in which both fungi and bacteria were associated with *Phytophthora infestans*. Murphy & McKay (1925) commented upon the two main types of rot and more recently Wilson & Boyd (1945) pointed out that the wet type of rot appeared to be invariably associated with *P. infestans*. In an investigation of the bacterial rots of stored potatoes, one of us (D.R.J.) noticed the same association in both field and laboratory examinations, and also that considerable amounts of liquid exuded from *Phytophthora*-infected tubers when kept in a saturated atmosphere. This seemed to be in accordance with the findings of Hagenuth & Griesinger (1941) that *Phytophthora*-infected tissue had a uniformly higher (average 2 %) water content than healthy tissue. When an infected tuber is cut in half and squeezed, appreciable amounts of liquid appear in the vicinity of the characteristic brown areas of the flesh: but in any naturally infected tuber numerous bacteria are always present in addition, and the question arises as to whether *Phytophthora* is solely responsible for the production of the liquid, or whether bacteria are also involved. At the instigation of Prof. Brooks, it was decided to infect sound tubers with *P. infestans*, and when the fungus was established to ascertain if liquid was produced; and if so, its amount and nature compared with that in normal tissue. It was also decided to inoculate such diseased tissue with various bacteria so as to compare their development with that of bacteria introduced into wounded but otherwise healthy tissue. Thus the investigation would be confined to the activities of not more than two organisms, the fungus and the bacterium, both in pure culture.

Experimental*

1. *Inoculation of healthy tubers with pure cultures of* P. infestans

Carefully selected sound tubers were washed to remove soil, immersed for 1 min. in methylated spirit, which acted as a wetting agent, and then immersed for 10 min. in a 0·1 % solution of mercuric chloride. Following this surface sterilization, they were washed in running tap water for 10 min. and immersed for a further minute in methylated spirit. The tubers were supported on sheets of glass resting on glass tripods over water in sterilized china incubation chambers with loose-fitting lids (Rudd Jones, 1950). The lids of the chambers were sealed by using pieces of split rubber tubing round the rims.

Three scratches approximately 2 mm. deep and 2·5 cm. long were made by a sterilized scalpel across the skin of each of five tubers, which were then inoculated with *P. infestans* in the following manner: a pure culture of the fungus was obtained from Mr S. Dickinson, and transfers were grown in wide tubes on slopes of wet maizemeal yeast-extract agar adjusted to pH 6·0. In order to provide a saturated atmosphere within the tubes, about 1 ml. sterile water was added to the sloped agar before inoculation. The production of conidia and zoospores took place within about 4 days. A few ml. of sterile water were added to make a suspension of conidia and zoospores. This was kept in the refrigerator for an hour at 5° C. and then exposed to room temperature at 18° C. for another hour to stimulate the discharge of zoospores.

The inoculated tubers were incubated at room temperature for 7 days, and were sprayed each day with sterile water to maintain a saturated atmosphere within the chamber.

Aerial mycelium and conidia then developed along the scratches, and on cutting the tubers, brown flecking of the tissues became apparent. The fungus was well established after 14 days, and when the tubers were cut and squeezed, they exuded much more liquid than uninoculated tubers kept under the same conditions.

Thus the presence of *P. infestans* was shown to be intimately associated with the production of liquid in diseased tissue.

2. *Inoculation of bacteria into tubers artificially infected with* P. infestans

Tubers infected with *P. infestans* as described in the preceding section were inoculated with bacteria by placing pieces of agar from a plate culture carrying a 48-hr. growth on to the infected areas of the tubers. Controls of *Phytophthora*-infected tubers and also healthy tubers were inoculated with pieces of sterile agar.

The pathogen, *Bacterium carotovorum* (strain P2J—Rudd Jones, 1950), and the saprophytes *Pseudomonas fluorescens* and *Bacterium rubefaciens*, a red, pigment-producing bacterium isolated from rotting tubers, were used in these inoculations.

After 10 days at 25° C. in a saturated atmosphere, the tubers were removed from

* The whole of the experimental work was done by D.R.J.

the incubation chamber, washed, surface sterilized in 0·1 % mercuric chloride and cut open with a sterile scalpel. Suspensions prepared from rotted tissue at various levels were plated out by the dilution method. *Bacterium carotovorum* was isolated 17 mm. below the point of inoculation, and at this depth the bacterium was spreading from the blight-infected zone and invading healthy tissues. *Pseudomonas fluorescens* was isolated in large numbers 7 mm. below the point of inoculation, and *Bacterium rubefaciens* had penetrated to a similar depth. Neither of the two latter organisms penetrated beyond the limits of *Phytophthora infestans*. In all the experiments the sound controls were not affected with the inoculated bacteria, but the blight-infected control tubers were all contaminated with secondary bacteria. Furthermore, the bacterial flora from the inoculated tubers always contained contaminants in addition to the inoculated bacteria, but they were never present in large numbers. It was impossible to avoid this contamination, as ideal conditions for secondary infection were presented by such long periods of incubation.

These experiments have confirmed the field observations of the development of secondary bacterial infection in blight-infected tubers subjected to a saturated, or nearly saturated, atmosphere. They have further shown that both parasitic (i.e. *Bacterium carotovorum*) and saprophytic (i.e. *Ps udomonas fluorescens* and *Bacterium rubefaciens*) bacteria can penetrate such tissue under these conditions, though neither type can infect undamaged healthy tubers.

3. *Nature of the liquid associated with* Phytophthora infestans-*infected tubers*

(*a*) *Sugar content of infected tissue*

Tubers of the varieties Arran Banner, King Edward and Duke of York were inoculated with *P. infestans*. The fungus was allowed to develop in a saturated atmosphere at room temperature (approximately 18° C.) for 4–5 days, and the tubers were then stored for a further 5–6 days at 85 % R.H. After removing the skin of the tubers, samples of tissue showing the early stages of the host reaction (i.e. a slight brown discoloration) and samples from similar positions in healthy tubers were cut out. The saps were extracted from both kinds of tissue in a hydraulic press and filtered through Seitz filters into sterilized flasks. 5 ml. samples of the filtrates were used for sugar estimations by the copper method of Narain (1932). The results are recorded in Table 1.

TABLE 1. *Concentration of sugars (sucrose and reducing) in sap extracted from healthy and infected tubers in g./100 ml. of extracted sap*

	Total sugars			Sucrose			Reducing sugars		
Variety	Healthy	Infected	Diff.	Healthy	Infected	Diff.	Healthy	Infected	Diff.
Arran Banner	0·35	0·68	0·33	—	—	—	—	—	—
King Edward	0·28	0·98	0·69	0·11	0·32	0·21	0·17	0·66	0·49
Duke of York	0·30	0·88	0·58	—	—	—	—	—	—

In all three varieties the sugar content was higher in sap from blighted tissue, thus confirming Lepik's (1929) observations. In King Edward, both the sucrose and reducing sugar content were higher. The results, recorded in Table 1, were calculated as g. of equivalent glucose/g. of sugar present in the extracted sap.

(*b*) *Osmotic pressure of sap extracted from healthy and blighted tubers*

This was estimated by two methods:

(i) Depression of freezing-point. Sap was extracted from healthy and artificially infected tubers of three varieties, Arran Banner, King Edward and Duke of York. Ice-salt freezing mixtures of about $-2°$ C. were used in a microcryoscope of the Walter (1931) type. About 5 ml. of the extracted sap was pipetted into a central tube, and with continual stirring was supercooled to about $-1{\cdot}5°$ C. when it was seeded with a small piece of nichrome wire cooled in liquid air. The true depression of freezing-point was calculated from the expression: Depression of freezing-point $D = D'\ (1 - U/80)$, where D' = observed depression and U = undercooling ° C. The results of the determinations are recorded in Table 2. In all three varieties the freezing-point was depressed more in sap from blighted than from healthy tubers, thus indicating a higher osmotic pressure in the former.

Table 2. *Depression of freezing-point of sap extracted from healthy and blighted tubers*

Variety and month	Healthy (° C.)	Blighted (° C.)
Arran Banner, Oct.	0·55	0·70
Arran Banner, Nov.	0·61	0·72
King Edward, Dec.	0·81	1·03
Duke of York, Dec.	0·42	0·70

(ii) Change in weight of tuber tissue immersed in sap extracted from healthy and blighted tubers. Blocks of tissue $2{\cdot}0 \times 2{\cdot}5$ cm. were removed from the outer tissue of healthy tubers with a cork-borer, and each was cut into five disks approximately 5 mm. thick. The disks were dried on filter-paper, and random samples of five disks were then weighed and immersed in the two saps for 1 hr. They were then removed, dried and weighed again. The results are recorded in Table 3. In all three

Table 3. *Comparison of osmotic pressure of sap extracted from healthy and blighted tubers, by loss in weight of samples of tuber tissue immersed in the two saps*

	Weight of disks (g.)			Weight of disks (g.)		
Variety and date	Before immersion in sap of healthy tissue	After 1 hr. immersion in sap of healthy tissue	Loss (g.)	Before immersion in sap of blighted tissue	After 1 hr. immersion in sap of blighted tissue	Loss (g.)
Arran Banner, Nov.	8·67	8·48	0·2	9·98	9·67	0·31
King Edward, Dec.	8·31	7·97	0·34	8·41	7·90	0·51
Duke of York, Dec.	9·02	8·74	0·28	8·42	7·99	0·43

varieties the percentage loss in weight was greater in those disks immersed in sap from blighted tubers than in sap from healthy tubers, indicating again that the sap from the blighted tubers had a higher osmotic pressure.

(*c*) pH *of the saps of healthy and blighted tissue*

Readings of the pH of the extracted saps were made using colorimetric methods and a pH meter (with glass electrode). Bromo-thymol blue was used as an indicator with a B.D.H. capillator, but some difficulty was experienced in obtaining good colour comparisons since the colour of the extracted saps masked that of the indicator. The saps, which are straw-coloured when first extracted, quickly darken on standing.

The readings are recorded in Table 4. The results with the colorimetric method tended to be higher than those obtained with the pH meter; but in all three varieties, the sap from infected tissue had a higher pH than that from healthy tissue, thus confirming the observations of earlier workers.

TABLE 4. pH *of saps extracted from healthy and blighted tubers*

Variety	Method	pH healthy	pH blighted
Arran Banner	Colorimetric	6·8	7·4
Arran Banner	pH meter	6·13	7·12
King Edward	Colorimetric	6·5	7·0
King Edward	pH meter	5·94	6·69
Duke of York	pH meter	6·18	6·73

DISCUSSION

The presence of large quantities of free liquid in the *Phytophthora*-infected tissue of tubers has been observed both in the field and in laboratory experiments, and the evidence from commercial and experimental clamps indicates that the atmosphere normally in contact with stored tubers has a relative humidity of about 95 %. The problem has been to determine the conditions under which infected tissue provides a suitable environment for the establishment of secondary bacteria.

The results of experiments with extracted sap are in agreement with those of Lepik (1929) and show that both the sugar content and osmotic pressure are higher in blighted than in healthy tissue. If this change takes place before the death of the infected cells, it is suggested that the suction pressure of these cells will be increased and water will be drawn from the adjacent healthy cells. The fungus eventually kills the host cells and the cell sap diffuses through the infected tissue.

In a blighted tuber the liquid accumulates in the infected tissue, and in an atmosphere near to saturation does not readily evaporate. With the release of cell sap an ideal medium is provided for the establishment of secondary bacteria. Abundant moisture together with a readily assimilable carbon source in the form of sucrose and reducing sugars, nitrogen compounds, and a pH near to neutrality, are available for the growth of bacteria. The preponderance of a particular species

depends upon the amount of inoculum, together with the ability to compete with other organisms of similar growth requirements.

The junior author (D.R.J.) wishes to express his thanks to the Agricultural Research Council for a special research grant to carry out this work, and both authors are indebted to Professor F. T. Brooks for his interest.

REFERENCES

Delacroix, G. (1903). Sur la pourriture des pommes de terre. *Bull. Soc. mycol. France*, **19–20**, 356.

Hagenuth, K. & Griesinger, R. (1941). Untersuchungen über den Stickstoffsausalt der Kartoffelknolle bei der *Phytophthorafäule*. *Phytopath. Zeit.* **13**, 517.

Lepik, E. (1929). Untersuchungen über den Biochemismus der Kartoffelfäulen. I. Der Einfluss der *Phytophthorafäule* auf die chemische Zusammensetzung der Kartoffelknolle. *Phytopath. Zeit.* **1**, 49.

Murphy, P. A. & McKay, R. (1925). Further developments on the sources and development of blight infection in potato tubers. *J. Dept. Agric. Ireland*, **25**, 10.

Narain, R. (1932). Carbohydrate metabolism in respiring leaves of *Hedera helix*. Ph.D. Thesis, University of Cambridge.

Rudd Jones, D. (1950). On the nomenclature and identity of the coliform soft-rot bacteria. *Trans. Brit. mycol. Soc.* **33**, 73.

Walter, H. (1931). Die kryoskopische Bestimmung des osmotischen Wertes bei Pflanzen. *Handb. d. biolog. Arbeitsmeth.* **11** (4), 353.

Wilson, A. R. & Boyd, A. E. W. (1945). Potato wastage in clamps. *Agriculture*, **51**, 507.

(*Received* 16 *June* 1950)

STUDIES IN BACTERIOSIS, XXVII

FACTORS INFLUENCING INFECTION BY *CORYNEBACTERIUM FASCIANS* (TILFORD) DOWSON

By S. E. JACOBS and U. MOHANTY

Bacteriological Laboratory, Imperial College of Science and Technology, London

(With Plate 3)

Laboratory experiments showed that gram (*Cicer arietinum*) could be infected by *Corynebacterium fascians*. Parallel tests on sweet peas and gram confirmed the existence of host specificity among strains of the pathogen, and of differences in host susceptibility towards a given strain. Adventitious root formation appeared as a new, though rare, sign of infection. Inoculation of aerial dormant buds invariably caused infection whereas cotyledonary bud inoculation often failed. Sporing bacteria antagonistic to *C. fascians* in culture media were isolated from soil, but they failed to exert their influence in infection experiments in sand.

Introduction

Corynebacterium fascians (Tilford) Dowson has a wide host range. It causes the well-known 'leafy-gall' and 'cauliflower' diseases, and has also been isolated from plants suffering from the 'dwarfing' disease (Lacey, 1948). However, although the disease is widespread, it is sporadic in its occurrence. Except under horticultural conditions there is rarely a high local incidence of infection, and apparently in the field it does not readily spread from affected plants. In laboratory experiments Tilford (1936) found the average infection of sweet peas to be only 64 %, the seeds being inoculated at sowing time. On the other hand, Lacey (1936) demonstrated that the age of the sweet-pea seedling at inoculation was most important, 100 % infection with severe symptoms being obtained in seeds inoculated just after they had germinated. Susceptibility fell off markedly as inoculation was delayed. Further, Lacey (1939) showed that amongst the families of plants, genera, species, and even varieties differed in susceptibility. The particular strain of *C. fascians* investigated may also be of importance, since occasionally it was found that all the colonies tested in an isolation failed to infect sweet peas, while a strain pathogenic for sweet peas and chrysanthemums might fail to infect *Pisum sativum* or *Vicia faba* (Lacey, 1939). All this suggests that, while the pathogen is of common occurrence in the soil, factors are at work to limit its ability to infect. Among these factors, host susceptibility and strain specificity have already been indicated. It may be pointed out that *Corynebacterium fascians* is Gram-positive and therefore belongs to the group of bacteria most sensitive to antibacterial agents, including antibiotics. The activities of the other members of the soil flora, many of which are known to form

antibiotics in culture media, may thus affect the outcome when a seedling or cutting of a susceptible plant is placed in infected soil. Further, *C. fascians* can attack the plant only where dormant buds exist, so the number of infection sites is limited. The experiments described below were undertaken with the object of confirming and extending earlier observations and of throwing light on some of the factors involved in the establishment of infection by *C. fascians*.

Materials and methods

Strains of Corynebacterium fascians *employed*

Twenty-two strains of *C. fascians* were used. Their origin and characters have been described elsewhere (Mohanty, 1951), but here it may be stated that half of them (Strains 1 S to V_{10}S inclusive, see Table 1) were recent isolations made by Mohanty between May and October 1946. The remainder had been isolated by Dr M. S. Lacey at various dates between 1935 and 1945. The letters S and R attached to the strain numbers indicate the culture phase, smooth or rough. All rough variants appeared during laboratory cultivation, the strains being smooth when first isolated. Strain 2WR was a white mutant of strain 2S.

Seed

Tests have been made on both sweet peas and the Indian pulse known as 'gram' (*Cicer arietinum*). The former, labelled 'mixed-hybrids', was of poor quality, with a low percentage germination—this work having been performed shortly after the war. The gram was a pure line known as 'Punjab 7'.

Seed sterilization and germination

This was effected by immersion in absolute alcohol for 2–3 min., followed by 1:1000 mercuric chloride for 10 min. After three rinses in sterile water the seeds were transferred to sterile Petri dishes lined with moist blotting paper and kept at 25° C. for 7 days, when the plumules were about 1 cm. long.

Cultivation of seedlings

The sweet pea inoculations were performed on seedlings germinated as above and placed singly in 6 × 1 in. plugged tubes containing about 12 g. of moist sand, the tubes and sand having been sterilized by autoclaving before the seedling was inserted with aseptic precautions. No additions of nutrients were made except where stated.

Inoculations of gram were made on seedlings planted in sterile moist sand in 500 ml. beakers, at the rate of twenty per beaker. The change in technique was solely because of limited space in the greenhouse in which all the seedlings were subsequently placed.

Methods of inoculation

Where basal infection only was required, as evidence of the pathogenicity of the strains, single sweet-pea seedlings in tubes were inoculated by adding 0·1 ml. of a visibly turbid suspension in sterile water of the culture to be tested. This was prepared by emulsifying growth from a culture on nutrient agar. The groups of twenty gram seedlings in beakers were inoculated more heavily, about 5 ml. of suspension being added. In all these cases inoculation was made immediately after the germinated seedlings had been placed in the tubes or beakers. Controls all received sterile water only.

In testing for ability to infect at internodes or leaf axils, soil sterilized by autoclaving was heavily infected with the pathogen and the mixture smeared over the stem or caused to lodge in the axil. Sometimes the tissue was also lightly scarified with a fine needle. The inoculated parts were covered with sterile absorbent cotton which was kept constantly moist with sterile water. Controls were similarly treated, using sterilized soil only.

Criterion of infection

Observations were made at intervals for 4 weeks in all cases, when multiple bud development was well advanced in infected seedlings, while non-reactors showed no abnormalities. An earlier sign of infection was the stimulation into growth of the dormant bud. This took as a rule 10–17 days. Occasionally, adventitious root formation was observed, either alone or accompanying fasciation.

Antagonistic organisms

These were obtained by the method of Waksman (1945). A 1 : 5,000 or 1 : 10,000 dilution of soil was heavily infected with *Corynebacterium fascians* and the mixture inoculated liberally on to the surface of nutrient agar plates. After incubation, colonies of organisms antagonistic to *C. fascians* were seen to be surrounded by zones in which the background growth of the latter organism had been inhibited. A number of such cultures was obtained, from both infected and presumably uninfected soil. All proved to be strains of either *Bacillus subtilis* or *B. mesentericus*.

Results

The infective capacities of twenty-two strains of Corynebacterium fascians

In Table 1 the results are recorded of trials of the infectivity of all the twenty-two strains of *C. fascians* on sweet-pea and gram seedlings, using the method of inoculation without wounding by means of suspensions in sterile water.

With seven of the strains none of the fifteen sweet-pea seedlings inoculated showed fasciation. Three of these strains were recent isolations, though not from sweet peas. Strain S5S came from an unusual specimen (Pl. 3, fig. 1), a 'cauliflower' on a garden pea, and was only isolated with great difficulty. H8S was from *Heuchera* and A9S

rom *Antirrhinum*, both typical specimens. All three were culturally and biochemically typical strains of *Corynebacterium fascians* (Mohanty, 1951). The other four strains were old cultures which might have deteriorated during laboratory cultivation, though when first isolated they were very infective, and they were known to have retained their pathogenicity during regular transfers on laboratory media for at least 5–6 years after isolation (Lacey, private communication). Repeated trials on sweet peas have been made with these seven strains but always with negative results. It would seem that the ability to infect this host was either completely lost or never present.

In the trials on gram the number of plants varied because twenty seedlings proved rather too many in a 500 ml. beaker and plants were lost through a root rot which tended to develop, with blackening of the tissue. The records are based on the number of plants surviving after 4 weeks.

It is clear that successful inoculations were obtained. *Cicer arietinum* is a new host plant for *Corynebacterium fascians*. Of the thirteen strains infective for gram all except two (12S and 12R) also attacked sweet peas. These two strains were originally infective for sweet peas, and have therefore lost the ability to attack one host while still possessing the ability to attack another. Nine strains were unable to cause fasciation of gram. These included the three recent isolations which failed to infect sweet peas, together with another, V10S, which had given good results on sweet peas. The five other non-infective strains were all old cultures. Two, 421S and 405R, had also failed against sweet peas, though infective for them when isolated. The other three had proved quite active on sweet peas, though not originally sweet pea strains. It is also clear that where the S and R variants of the same strains were tested (2, 12, 484 and 496), they behaved alike towards a given host, both being infective or both non-infective.

In no case was 100 % infection obtained with sweet peas, the highest figure being 67 %. With gram, maximum infection occurred in one case, but this record is rather unsatisfactory because of the presence of the root rot referred to above. The strain concerned, P7S, was one which caused an unusually large number of basal shoots to develop, and these grew to a considerable extent (Pl. 3, fig. 2).

When sweet peas are grown in sand cultures under the conditions described, adventitious root formation is very rare. However, in certain cases in the present work, adventitious roots appeared in inoculated plants from the region of the stem not far above the junction with the root. The four strains concerned are marked with asterisks in Table 1. Two of them, H8S and A9S, failed to induce fasciation, while the others, 2S and V10S, caused fasciation in addition (Pl. 3, figs. 3 and 4). The reaction is not specific to sweet peas, since strain 2S caused root formation in gram also, the appearance being illustrated in Pl. 3, fig. 5. Adventitious root formation has been repeatedly encountered in experiments other than that described above, but its occurrence has been relatively infrequent.

TABLE 1. *The production of fasciation on sweet-pea and gram seedlings by various strains of* Corynebacterium fascians

Strain	Original host	Sweet pea		Gram	
		No. of plants	No. fasciated	No. of plants	No. fasciated
1S	*Dahlia*	15	8	20	16
2S	Wallflower	15	9*	10	6*
2WR	Wallflower	15	10	15	6
3S	*Primula*	15	6	20	7
P4S	*Phlox*	15	7	7	3
S5S	Garden pea	15	0	15	0
V6S	*Verbascum*	15	8	16	10
P7S	*Pelargonium*	15	9	10	10
H8S	*Heuchera*	15*	0	12	0
A9S	*Antirrhinum*	15*	0	10	0
V10S	*Verbascum*	15	8*	8	0
12S	Sweet pea	15	0	10	5
12R	Sweet pea	15	0	5	2
484S	Strawberry	15	7	8	5
484R	Strawberry	15	8	5	2
421S	*Asparagus*	15	0	10	0
405R	*Nicotiana*	15	0	8	0
448S	*Petunia*	15	9	8	0
444S	*Chrysanthemum*	15	7	10	2
496S	Wallflower	15	10	10	0
496R	Wallflower	15	6	8	0
497S	*Primula*	15	5	10	3

* In a few of these plants adventitious roots appeared.

Infection of the aerial parts of sweet-pea plants

Strain 2S was used exclusively in these experiments. Controls were always set up, and proved consistently negative.

(i) *Infection at internodes*

Twenty plants were inoculated with infected soil by the method described, half being wounded also. In no case did any sign of disease develop even after 6 weeks. There was no swelling at the site of inoculation, and no stimulation into activity of the neighbouring or other axillary buds. Wounding therefore had no effect in facilitating infection, and when the experiment was terminated it was seen that the wounds had healed.

(ii) *Infection at foliage-leaf axils*

Twenty plants were inoculated, each at a single axil, with infected soil, half being wounded. In all cases, irrespective of wounding, the dormant bud was stimulated and development became evident in 10–17 days. After 20–30 days multiple bud development was well advanced. Axils other than those treated did not respond. The effect obtained is illustrated in Pl. 3, fig. 6.

(iii) *Infection at scale-leaf axils*

Sweet-pea plants usually bear two scale leaves below the first foliage leaf. In normally grown plants the internode between the cotyledons and the first foliage leaf is short, and the scale leaves close together. By growing the plants in the dark for a time the internode was caused to elongate, making it easier to locate the scale leaves and inoculate them separately from the cotyledonary or foliage leaf axils. Five such plants were inoculated at the scale leaves, without wounding, by the infected soil method. In all cases dormant buds in the scale leaf axils had begun to grow within 15–20 days, and scale leaves therefore behave like foliage leaves. The appearance obtained is shown in Pl. 3, fig. 6.

(iv) *Simultaneous infection of two axillary buds on a plant*

The sweet-pea plants grown as described usually bore three or four leaves apart from the apical bud. In attempts to inoculate in the axils of the upper foliage leaves the apical bud was often damaged. This in itself would induce development of axillary buds, so attention was confined to the axils of the two lower leaves. Inoculations at both these axils led to simultaneous development of both dormant buds, as shown in Pl. 3, fig. 7.

A further point may be made, namely that infection invariably occurred when inoculation with strain 2S was made in a leaf axil. This contrasts with the situation when the inoculum was applied to the sand beneath the germinated seed, where 67 % infection only was obtained.

Infection in the presence of antagonistic bacteria

Corynebacterium fascians, as a Gram-positive organism, belongs to the class of bacteria most sensitive to antibacterial agents, and in fact strains of *Bacillus subtilis* and *B. mesentericus* able to produce substances inhibitory to its growth were readily isolated from soil by the method described above. The inhibition of strain 2S by a culture of *B. mesentericus* is illustrated in Pl. 3, fig. 8. These bacteria may perhaps play a part in determining whether a plant shall or shall not become infected from the soil. To test this, young sweet-pea seedlings were inoculated without wounding with mixtures of *Corynebacterium fascians* 2S with either the actively antagonistic *Bacillus subtilis* or *B. mesentericus*, inoculation with *Corynebacterium fascians* alone forming the control. The results obtained after the usual four-week period are given in Table 2, which embodies the results of two trials. The results were consistent, and indicate that in the presence of *Bacillus mesentericus* the incidence of infection was only about half that in the control. *B. subtilis* had no effect.

It is common experience that antibiotic production occurs best in rich media, while in the experiments just described nutrients for the antagonistic bacteria were virtually absent. It was thought that more positive results might have been achieved if food had been present. An experiment was therefore performed in which such an

TABLE 2. *The influence of antagonistic bacteria on the percentage infection by strain* 2S *of sweet-pea seedlings in sand cultures*

Antagonistic organism		No. of plants inoculated	Plants fasciated (No.)	Plants fasciated (%)
Bacillus subtilis	(*a*)	10	5	50
	(*b*)	30	17	57
B. mesentericus	(*a*)	10	3	30
	(*b*)	30	8	27
Control	(*a*)	10	6	60
	(*b*)	30	15	50

addition was made, but in order not to make the conditions too much unlike those in soil, the added nutrients were in the form of soil extract. This was prepared by autoclaving a mixture of 500 g. of soil with 500 ml. of tap water plus 0·5 g. of sodium carbonate for 15 min. at 15 lb. pressure. The filtrate from this was added at the rate of 0·5 ml./tube, the tubes sterilized, and the seedlings and bacterial suspensions added. Thirty replicates were set up in each treatment. The results, which are given in Table 3, show that the percentage infection was higher than before in the control, and it was observed that the signs of disease appeared rather earlier. It is not known whether the increase is significant, but it may indicate stimulation of *Corynebacterium fascians* by the nutrients added.

TABLE 3. *The influence of antagonistic bacteria on the percentage infection by strain* 2S *of sweet-pea seedlings in sand plus soil extract*

Antagonistic organism	No. of plants inoculated	Plants fasciated (No.)	Plants fasciated (%)
Bacillus subtilis	30	25	83
B. mesentericus	30	26	87
Control	30	23	77

With the antagonists present also, the percentage infection was if anything higher than in the control, and clearly the right conditions for the demonstration of antagonism against infection were not secured. Either antibiotic substances were not produced, or, if they were, stimulation of the pathogen by the nutrients nullified their action.

DISCUSSION

Lacey (1948) has stressed that the signs of the 'leafy-gall' disease point to a profound disturbance of the normal auxin mechanism in infected plants. There are many puzzling features about this disturbance, and much remains to be done before a clear picture of the action of the pathogen on its host can be obtained. On current theories, the stimulation into activity of dormant buds could be ascribed to a reduction in the auxin concentration at those sites, while the marked thickening often seen

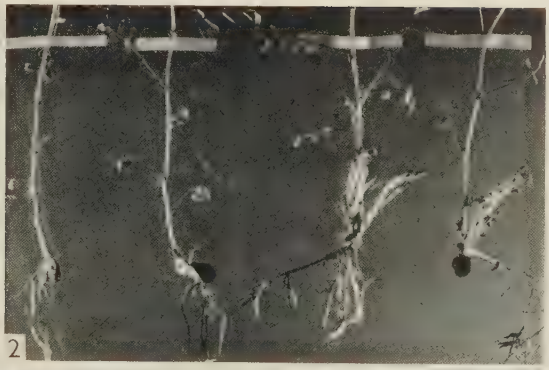
in the stimulated shoots indicates the presence in them of excessive quantities of auxin. These requirements seem at first sight to be mutually exclusive, though circumstances may be envisaged by which this situation could arise. Adventitious root formation is a response commonly observed when heteroauxins are applied to plant stems, and the production of such roots in infected seedlings is suggestive, though at present it can only be taken as one more indication of the disturbed state of the auxin regulatory mechanism.

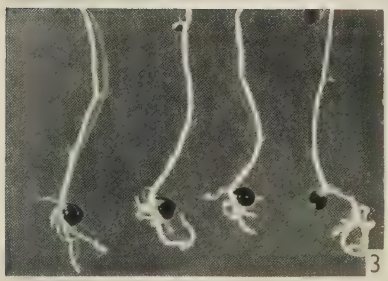
The present work has yielded evidence in support of the conclusion reached by Lacey (1939), that there may be both strain specificity and differences in host susceptibility. Nevertheless, the degree of infection obtained when seeds were inoculated shortly after germination rarely reached 100 %, and was often much less. This may have been due in the case of the sweet peas to the use of non-homogeneous material, though a pure line of gram was used. This may, however, not have been pure in respect of infectibility.

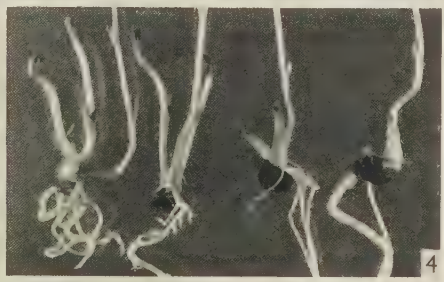
A noteworthy feature of the work was that infection invariably occurred when suitable aerial parts of the plants were inoculated. This suggests that the factors which operate to prevent the realization of 100 % infection when freshly germinated seeds are exposed to the pathogen are not operative when the alternative technique is used. Now the same mixture of host-plant varieties was used in each case, so the tissue itself could not have been the cause of the difference, but it is obvious that in inoculation of aerial parts there is no contact with the sand, into which substances may have diffused from the seed, and in which contaminating micro-organisms may have been growing. No examination was made of the sand in the culture tubes to see whether contamination had occurred, but it was shown that *Corynebacterium fascians* is very susceptible to antibiotics formed by soil bacteria, and these may have had some effect in reducing the percentage infection. Although when organisms known to be antagonistic were introduced the effect could not be demonstrated, this may have been due to the use of unsuitable types of antagonists, and the point cannot be regarded as settled. On the subject of substances diffusing from the seed into the sand, these may be of two kinds, stimulatory or inhibitory to *C. fascians*. It is known that antibiotics do frequently occur in plants, including the seeds, and it may be that the presence of these in certain seeds may determine whether infection is or is not to occur in the case of a given strain of the pathogen. It is possible also that strains unable to cause infection when tested in the usual manner may be able to attack successfully when inoculated on to dormant buds in the aerial parts of the plants. Work is now proceeding on these aspects of the problem.

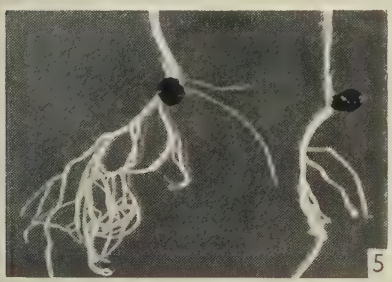
The participation of U. Mohanty in this work was made possible by the award of a Scholarship from the Government of India, to whom grateful thanks are due.

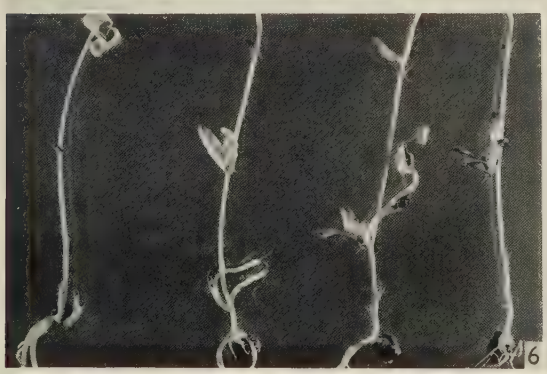
















JACOBS and MOHANTY—*Studies in bacteriosis, XXVII*

REFERENCES

LACEY, M. S. (1936). Studies in bacteriosis, XXII. I. The isolation of a *Bacterium* associated with 'fasciation' of sweet peas, 'cauliflower' strawberry plants and 'leafy-gall' of various plants. *Ann. appl. Biol.* **23**, 302.

LACEY, M. S. (1939). Studies in bacteriosis, XXIV. Studies on a bacterium associated with leafy galls, fasciations and 'cauliflower' disease of various plants. Part III. Further isolations, inoculation experiments and cultural studies. *Ann. appl. Biol.* **26**, 262.

LACEY, M. S. (1948). Studies on *Bacterium fascians*. V. Further observations on the pathological and physiological reactions of *Bact. fascians*. *Ann. appl. Biol.* **35**, 572.

MOHANTY, U. (1951). *Corynebacterium fascians* (Tilford) Dowson; its morphology, physiology, nutrition and taxonomic position. *Trans. Brit. mycol. Soc.* **34**, 23.

TILFORD, P. E. (1936). Fasciation of sweet peas caused by *Phytomonas fascians* n.sp. *J. agric. Res.* **53**, 383.

WAKSMAN, S. A. (1945). *Microbial Antagonisms and Antibiotic Substances*. New York: The Commonwealth Fund.

EXPLANATION OF PLATE 3

Fig. 1. Naturally infected garden pea with an aerial cauliflower-like gall.

Fig. 2. Artificially infected gram seedlings (*Cicer arietinum*) with basal fasciation consisting of numerous and unusually long shoots. Strain P7S. Uninoculated plant on the left.

Fig. 3. Adventitious root formation without fasciation, induced in sweet-pea seedlings by strain A9S. Uninoculated plant on the right.

Fig. 4. Adventitious root formation with fasciation, induced in sweet-pea seedlings by strain 2S.

Fig. 5. Adventitious root formation with fasciation, induced in gram seedlings by strain 2S. Uninoculated plant on the right.

Fig. 6. Stimulation of dormant buds in sweet-pea seedlings by strain 2S. Left to right, cotyledonary buds, scale-leaf bud and two foliage-leaf buds. Only the inoculated axils have responded.

Fig. 7. Simultaneous stimulation of two axillary buds on the same stem of a sweet-pea seedling, following inoculation of both axils with strain 2S. On the right, only one axil inoculated.

Fig. 8. Zone of inhibition of *Corynebacterium fascians* strain 2S around the growth of a culture of *Bacillus mesentericus*.

(*Received* 6 *June* 1950)

THE EFFECTIVENESS OF STRAINS OF THE NODULE ORGANISM WHEN ASSOCIATED WITH DIFFERENT SPECIES OF CLOVER

By G. BOND and M. P. McGONAGLE

*Botany Department, University of Glasgow**

The effectiveness in nitrogen fixation of fifty-four strains of the clover nodule organism has been tested on red, white and alsike clover in aseptic tube-culture by comparing the nitrogen content of nodulated plants grown on nitrogen-free medium against that of non-nodulated plants grown on media with and without nitrate-nitrogen. The performance of the strains was to a considerable degree consistent on all three hosts. Minor changes were shown but for the most part did not exceed the experimental error. Thus in respect of these three clovers there is no marked host species effect. Seed inoculants or naturally occurring strains effective on one species are likely to be effective on the other species.

The study of the effectiveness of given strains of the nodule organism when associated with different species or genera of host plant is of interest in connexion with the general question of the importance of the host plant in determining effectiveness and also with the selection of strains for the field inoculation of seed. Studies of this type in the genus *Trifolium* have been carried out by workers in Australia. Strong (1937, 1940) reported that strains which were effective with red clover (*Trifolium pratense* L.) and white clover (*T. repens* L.) were ineffective with subterranean clover (*T. subterraneum* L.), and vice versa. Jensen & Vincent (1941) reported that this effectiveness pattern did not always hold, and envisaged the possibility of finding, for use in seed inoculation, a strain effective with all the common clovers. Purchase & Vincent (1949), in a more extensive survey, tested some 230 strains of the nodule organism on red, white, subterranean, and ball clover (*T. glomeratum* L.) About half of the strains behaved consistently on all four hosts. The performance of the remaining strains varied on different hosts, but there was a tendency to concurrence of result within the two host-pairs—red and white clover, subterranean and ball clover. In consequence seed inoculation was recommended when red or white clover is being introduced into an area where the other species have previously been cultivated.

In the present study the effectiveness of strains of the clover organism has been compared when associated with three of the more important clovers in this country, namely red, white and alsike clover (*T. hybridum* L.). Thornton (1946) has reported the relative frequency of ineffective isolations from clover plants of hill pastures in the north and west of Britain, and has referred to the inoculation of seed with

* The second author is now of the Botany Department, University of Manchester.

selected strains of the nodule organism as one method of improving the growth of clover in such areas.

Methods

With one exception the strains employed had been isolated in another connexion from field plants of clover collected from various districts of Western Scotland. Of the fifty-three local strains, forty-seven were originally isolated from white clover and the remainder from red, each isolation being from a different plant.

The test-plants were grown aseptically in test-tubes, a method employed by a number of previous workers, the tubes employed being $6 \times \frac{3}{4}$ in. and containing 10 c.c. slopes of the nitrogen-free agar medium of Chen & Thornton (1940), of initial pH 6·6. Commercial samples of seed were employed, and after surface sterilization four to five seeds were sown per tube. Subsequent to germination the number of plants was reduced to two per tube, and an appropriate inoculum added. Six replicate tubes were set up for each strain of nodule organism on a given host species. After 3 months' growth the dry weight and total nitrogen (Kjeldahl) of the plants were determined, the twelve plants normally being bulked together for these purposes. During the growth period the pH of the agar medium fell to values in the region of 5–5·5 in tubes containing plants associated with effective strains.

Evidence of the satisfactory control of the nodule organism is provided by the non-development of nodules in any of the seventy-two uninoculated control tubes with nitrogen-free medium set up in connexion with the trials. Occasional contamination with other micro-organisms occurred, but affected considerably less than 1 % of the total tubes.

The relative nitrogen contributions of the different bacterial strains were assessed against the nitrogen intake of uninoculated plants in culture media with adequate amounts of potassium nitrate, the nitrate being supplied at two levels (equivalent to 4 and 6 mg. nitrogen per tube), with twelve tubes at each level. Since the smaller amount of nitrogen gave slightly superior growth, the data for plants at this level were used for calculation of effectiveness. This was carried out in the manner suggested by Boyes & Bond (1942) by expressing the final nitrogen content of plants associated with a given strain as a percentage of that of the nitrate-fed plants, after deducting from both values the nitrogen content of uninoculated control plants.

On the basis of values calculated in this way, strains were arbitrarily classified as follows:

Effective. Where the value exceeded 70 %.

Ineffective. Where the value was less than 20 %.

Intermediate. Where the value lay in the range 20–70 %.

Most of the data were obtained in the summers of 1948 and 1949. In some trials nitrate reference plants were not included, the behaviour of strains on such occasions being assessed against a particular effective strain taken as a standard. Since the performance of this standard strain against nitrate reference plants was known from

other trials, it was possible to relate strains to the nitrate plants through the standard strain.

The satisfactory control which the method of tube culture gives over the incidence of the nodule organism is, however, accompanied by marked restriction of growth owing to lack of space and probably of carbon dioxide (Knudson, 1916). In consequence of this the maximum dry weight attained in the present trials has been of the order of 30 mg./plant, only one-tenth of that of clover plants grown in the same greenhouse in open culture. The opportunities for growth are, however, undoubtedly adequate for the assessment of effectiveness, as is indicated by data below.

Results

In Table 1 typical primary data for various types of plants are presented, and indicate that effective strains produced plants showing dry weights and nitrogen contents of the same order as those supplied with nitrate, while the growth of plants associated with ineffective strains was frequently inferior to that of uninoculated plants in nitrogen-free medium, the result, no doubt, of the essentially parasitic behaviour of such strains.

Table 1. *Examples of primary data (all mg./plant) for various types of plants**

Host clover		Uninoculated plus nitrate		Inoculated effective strain (no. 49†)		Inoculated ineffective strain (no. 113)		Uninoculated N-free cultures	
		Dry wt.	N content	Dry wt.	N content	Dry wt.	N content	Dry wt.	N content
Red	Set A	33·3	1·12	27·2	0·92	7·5	0·13	12·0	0·17
	Set B	34·1	1·08	27·8	0·98	—	—	11·4	0·16
White	Set A	32·0	0·98	28·4	0·89	5·7	0·09	6·2	0·07
	Set B	32·0	1·06	33·4	1·02	—	—	6·7	0·08
Alsike	Set A	27·2	0·92	27·9	1·07	4·3	0·06	5·5	0·09
	Set B	27·8	0·98	29·4	1·09	—	—	6·2	0·09

* All the data except those for the ineffective strain on red clover were obtained in trials extending from April to June, 1949. Each value is a mean based on a set of six tube-cultures with two plants per tube.

† This strain was originally obtained from Dr H. G. Thornton.

Agreement between data for the duplicate sets of tubes available for the particular effective strain included in the table is less close than in the case of the nitrate plants. The chief reason for this is the plant-to-plant variation in nodulated cultures noted in previous work with clover (Vincent, 1944; Thornton, 1946), and shown by Nutman (1946) to be due, at least in some cases, to genetical differences in the host plant. Instances of this from the present trials are as follows:

Strain 119 on alsike. The strain was ineffective on nine out of the twelve test-plants, the mean dry weight of these plants being 5·1 mg. The three remaining plants were much stronger and showed a mean dry weight of 24·3 mg.

Strain 94 *on white*. The strain was effective on nine of the test-plants, the mean dry weight being 35·6 mg., but that for the three remaining plants was only 5·7 mg.

Statistical data on the likelihood of error in estimating effectiveness are available from more extensive tests with two particular effective strains, in this instance on red clover, for each of which six sets each comprising six tubes were employed. With one strain the mean of the six estimates of effectiveness was 87, the standard deviation of a single value being 5·1. With the second strain the corresponding figures were 79 and 4·7. Accordingly, in ninety-nine cases out of a hundred, the estimate of effectiveness based on the single set of tubes normally employed should be within approximately ±15 of the correct figure. The possible error with intermediate strains, which frequently give results as with strain 119 (above), is undoubtedly somewhat greater.

The general conclusion regarding reliability is that the estimates of effectiveness may err by one class, e.g. a strain shown as intermediate might eventually be classed as ineffective or effective if it were practicable to employ considerably more replicate tubes and to use several stocks of seed of each host species. There is, however, no reason to suspect that two-class errors are likely to be incurred, i.e. that a strain which is actually ineffective should, on the basis of the scale of test employed, be classed as effective, or vice versa. Therefore when the performance of a strain on different host species is being compared, a shift from one class to an adjacent one is not necessarily significant, but a shift from ineffective to effective or vice versa may be accepted with some confidence as evidence of a host species effect.

TABLE 2. *Frequency of strains grouped according to their effectiveness on the three host species**

On red clover	On white clover	On alsike clover	No. of strains
E	E	E	8
EI	EI	EI	2
I	I	I	14
E	E	EI	2
E	EI	E	6
E	EI	EI	3
EI	E	E	3
EI	E	EI	3
EI	EI	E	3
EI	I	EI	2
EI	I	I	4
I	EI	EI	1
I	I	EI	3
Total			54

* E, EI and I signify effective, intermediate, and ineffective respectively.

In Table 2 the effectiveness patterns of the strains of nodule organism on the three host species are shown, the method of presentation employed by Purchase & Vincent (1949) being adopted. Of the twenty-seven possible patterns, twelve would

involve effectiveness with one or more hosts and ineffectiveness with the remainder. It will be noted that not one such pattern is represented in the table. All the differences in performance were one-class differences, which are not necessarily significant. Twenty-four strains behaved consistently on all three hosts, over half of them being of ineffective type. A few strains came near to showing two-class differences, as indicated by the fact that if the lower limit for the intermediate class were to be raised from twenty to thirty, then two strains would be classed as ineffective on one host and effective on another.

Discussion

The above data indicate that in respect of these three species of clover there is no marked host species effect. A number of minor changes in effectiveness appeared to have occurred, but few of them definitely exceeded the experimental error. A different arrangement of the data is provided in Table 3, where the use of the χ^2 test shows

Table 3. *Influence of host species on strain performance**

Class	Frequency of strains when tested on: Red clover	White clover	Alsike clover
E	19	16	20
EI	17	15	16
I	18	23	18

* The value of χ^2 obtained from the table $= 1{\cdot}45$. The required value for $P = 0{\cdot}05$ with four degrees of freedom $= 9{\cdot}49$.

that at $P = 0{\cdot}05$ there is no significant variation in performance of strains in association with different hosts. Thus the apparent tendency to a reduced effectiveness in association with white clover can be attributed to experimental error. In any case it should be borne in mind that white clover is of smaller habit than the other species, and that in consequence the growth of the nitrate reference plants was probably slightly less restricted by the confined growing space than in the other species. This would have the effect of lowering the relative growth of the corresponding nodulated plants, the development of which at least in early stages is restricted by shortage of nitrogen.

The results are in agreement with those of Australian workers (p. 247 above) in showing that strains of the nodule organism tend to behave similarly on red and white clover. It is now clear that alsike clover can be added to the group. From the practical point of view a seed inoculant known to be effective with one of these species is likely to be reasonably effective with the others. Areas containing native effective or ineffective strains for one of the species should be correspondingly favourable or unfavourable for the other species as regards the nodule organism.

The authors are indebted to the Agricultural Research Council for financial assistance, to Dr H. G. Thornton for supplying information on technique in the

early stages of the work, and to Mr A. C. Crundwell for suggestions as to statistical treatment. Mr T. P. Ferguson gave technical assistance.

REFERENCES

Boyes, J. & Bond, G. (1942). The effectiveness of certain strains of the soya-bean nodule organism when associated with different varieties of the host plant. *Ann. appl. Biol.* **29**, 103.

Chen, H. K. & Thornton, H. G. (1940). The structure of 'ineffective' nodules and its influence on nitrogen fixation. *Proc. roy. Soc.* B, **129**, 208.

Jensen, H. L. & Vincent, J. M. (1941). Specificity between host plant and strain of *Rhizobium trifolii*. *Aust. J. Sci.* **3**, 169.

Knudson, L. (1916). Influence of certain carbohydrates on green plants. *Mem. Cornell agric. Exp. Sta.* **9**, 739.

Nutman, P. S. (1946). Genetical factors concerned in the symbiosis of clover and nodule bacteria. *Nature, Lond.*, **157**, 463.

Purchase, H. F. & Vincent, J. M. (1949). A detailed study of the field distribution of strains of clover nodule bacteria. *Proc. Linn. Soc. N.S.W.* **74**, 227.

Strong, T. H. (1937). The influence of host plant species in relation to the effectiveness of the *Rhizobium* of clovers. *J. Counc. sci. industr. Res. Aust.* **10**, 12.

Strong, T. H. (1940). Non-effective associations of nodule bacteria and legumes, with particular reference to the influence of the host plant. *J. Aust. Inst. Agric. Sci.* **6**, 14.

Thornton, H. G. (1946). In *Rothamsted Exp. Sta. Report for* 1939–45. St Albans.

Vincent, J. M. (1944). Variation in the nitrogen-fixing property of *Rhizobium trifolii*. *Nature, Lond.*, **153**, 496.

(*Received 22 June* 1950)

THE CONFIRMATION OF THE VARIETY *FABAE* KEAY OF *SCLEROTINIA TRIFOLIORUM* ERIKSSON

By A. R. LOVELESS, M.A., Ph.D.
*Botany School, Cambridge**

(With Plate 4 and 1 Text-figure)

There is confusion as to whether the *Sclerotinia* on *Vicia faba* is a normal strain of *Sclerotinia trifoliorum* as found on clover and related legumes or a distinct variety (var. *fabae*). Pathologically the two fungi appear to be the same, and field observations indicate that beans in the rotation may aggravate the incidence of clover rot. A re-investigation of the morphology of the two fungi is made to assess the status of var. *fabae*.

The two fungi could not be distinguished by apothecial characters or cultural behaviour. Ascospore measurements confirm the existence of *S. trifoliorum* var. *fabae*, although *S. trifoliorum* proper also occurs on beans. Var. *fabae* has not been found on any legume besides beans. Isolates from trefoil and sainfoin agree in spore size with *S. trifoliorum* on clover.

When isolates of *S. trifoliorum* are co-inoculated on the same Petri plate, they either do not react, or react to form a 'demarcation line'. Although a demarcation line is assumed to imply a genetic difference, non-reaction does not necessarily imply genetic identity. Non-reaction and the formation of a demarcation line also occur when var. *fabae* strains are co-inoculated together. When an isolate of *S. trifoliorum* is co-inoculated with an isolate of *S. trifoliorum* var. *fabae* an 'incompatibility line' is formed. Use can be made of this fact in identification.

On the basis of co-inoculations *S. trifoliorum* and *S. trifoliorum* var. *fabae* are both shown to constitute a diverse range of biotypes. Segregation of biotypes in the ascus as a result of heterokaryosis in the ascus-initial is demonstrated. The view is expressed that heterokaryosis is the mechanism underlying the production of new biotypes.

Introduction

The fungus *Sclerotinia trifoliorum* Eriksson is the cause of the well-known 'clover rot' disease of clover and related leguminous crops. The host that is most commonly attacked in Great Britain is broad red clover (*Trifolium pratense praecox*), but late-flowering red (*T. pratense serotinum*), alsike (*T. hybridum*) and white clover (*T. repens*) are also susceptible. The disease also occurs on trefoil (*Medicago lupulina*), lucerne (*M. sativa*), sainfoin (*Onobrychis sativa*) and vetches or tares (*Vicia sativa*). There is confusion, however, as to whether the *Sclerotinia* on field beans (*Vicia faba*) is the same fungus as that on clover and related forage crops. At one time the Sclerotinia disease of beans was usually referred to as *Sclerotinia sclerotiorum* (Lib.) de Bary and sometimes as *S. trifoliorum*. The fact that 'bean rot' does not normally develop

* Now at Rendcomb College, Cirencester, Glos.

before mid-March or early April, and is most conspicuous from April to June, is probably the reason why *S. sclerotiorum* was thought to be the causal agent. Under natural conditions the apothecia of this species develop in spring and early summer, whereas those of *S. trifoliorum* develop in the late autumn. On the other hand, in certain heavy land districts of East Anglia, farmers have long been aware that clover should not be sown under wheat in a bean tilth, because this practice often leads to failure from *Sclerotinia*. Amos (1918) commented on this fact and in an uncontrolled experiment showed that it was possible to infect beans by placing under them apothecia grown from sclerotia formed on clover plants. This demonstration of the possible relationship of *S. trifoliorum* to bean rot was not followed up, and the identity of the causal organism remained uncertain.

It was not until 1939 that Keay (1939) finally established the relationship of *S. trifoliorum* to bean rot by mycelial cross-inoculation, by a consideration of host range, and by a comparison of the morphology of the apothecial fructifications and of the size of the asci and ascospores. Using bean isolates from four localities, Keay showed that they were distinct from *S. sclerotiorum* and essentially similar to *S. trifoliorum*. There were, however, slight but *constant* differences (Table 1) in morphological and cultural characters between the fungus on beans and that on clover, and it was proposed on these grounds that the bean fungus should be regarded as a definite variety. Keay suggested the name *S. trifoliorum* Erikss. var. *fabae* Keay, and this name has been adopted.

TABLE 1 (*after Keay*). *The differences between* Sclerotinia trifoliorum *and* S. trifoliorum *var.* fabae

	Sclerotinia trifoliorum	*Sclerotinia trifoliorum* var. *fabae*
Average diameter of apothecial disk	3·9 mm.	2·8 mm.
Average size of asci	180·10 × 13·55 μ	210·54 × 16·87 μ
Average size of ascospores	15·23 × 8·67* μ	19·02 × 9·97 μ
Time needed for mycelium to reach a diameter of 6 cm. at 20° C.	65·5 – 69·0 hr.	59·5 hr.

* This figure was published in error as 8·17.

If Keay is correct in maintaining that the *S. trifoliorum* attacking *Vicia faba* is distinct from that attacking clover and other leguminous crops, then the presence of *Sclerotinia trifoliorum* in a bean crop will not provide a source of inoculum for other legumes, and vice versa. Hence in a sequence of cropping, clover rot and the Sclerotinia disease of beans can be regarded as different diseases.

This view is difficult to reconcile, however, with certain circumstantial evidence that beans in the rotation may aggravate the incidence of clover rot. The following example (Moore, 1948) is typical of the evidence that has accumulated on this point. In 1943 at Kirton, Lincolnshire, there was a clear-cut line in one field between a seeds mixture under beans, and the same mixture sown on the same day under

oats. The red clover under the beans failed from clover rot, that under the oats gave a good crop with very little rot in it.

In order to establish the host relationships of the *S. trifoliorum* on clover and that on beans it is necessary to cross-inoculate the two fungi. Keay (1939) carried out this experiment on a mycelial basis and obtained positive results. Mycelial inoculation, however, is not necessarily demonstrative of pathogenicity in the field if infections arise afresh each year by ascospores. For this reason Dillon Weston, Loveless & Taylor (1946) repeated the experiment using ascospores. They showed conclusively that bean and clover plants could be infected, under high relative humidity, by ascospores from sclerotia on the opposite host.

These results therefore suggest that clover rot and the Sclerotinia disease of beans cannot be considered as two separate diseases in the field. On this assumption, field observations such as that recorded at Kirton find a ready explanation. It is not possible to reduce the var. *fabae* of *S. trifoliorum* to a synonym on the basis of similar pathogenicities under experimental conditions, because var. *fabae* was erected on purely morphological grounds. If *S. trifoliorum* var. *fabae* had been shown to be non-pathogenic to clover, i.e. to be ecologically differentiated from *S. trifoliorum*, there would have been every justification for its erection even on slight morphological differences. In the absence of any definite ecological distinction, however, it seemed necessary to re-investigate the morphology of the two fungi in order to assess the status of var. *fabae*. For this purpose three sets of characters have been studied, namely, apothecial characters, the size of the ascospores, and cultural characters.

Apothecia

(1) *Production of apothecia*

(*a*) *Methods and observations*

Under natural conditions the apothecia of *Sclerotinia trifoliorum* are recorded as developing chiefly in the late autumn, but continuing to emerge until early spring if the season is mild and damp. In the present work apothecia were produced from September to March, the main crop appearing each year during October and November. Similar times of apothecial production applied to isolates of *S. trifoliorum* from *Vicia faba*.

No mature apothecia of this species were produced in culture, although on several occasions apothecial stipes developed. The majority of these stipes shrivelled when only a few mm. in length, but some persisted to produce swollen, urceolate tips; in no instance, however, was a functional disk formed. The method used for obtaining apothecia was to bury sclerotia in soil. Flower-pots, with their drainage holes covered with wire gauze to exclude earthworms, were three-quarters filled with a medium loam, which was packed in tightly so as to avoid later subsidence; sclerotia were placed on the surface and then covered to a depth of $\frac{1}{2}$–$\frac{3}{4}$ in. by the

addition of more soil. The pots were kept outdoors, and were not watered except when dry weather persisted during the period of apothecial production.

The functional life of an apothecium may be equated with the period of ascospore discharge, which begins as soon as the disk is visible and long before expansion to the typical funnel-shape takes place. The duration of ascospore discharge is a variable quantity, depending largely on the size of the apothecium and on the weather, but on the average it is about 4 weeks.

An interesting feature in the production of apothecia from sclerotia of *Sclerotinia trifoliorum*, is that under no single set of soil conditions are all the sclerotia in a population likely to germinate. Several pots containing sclerotia that were formed under apparently identical cultural conditions and buried at the same time, have been kept under observation for three consecutive years. In the first autumn following burial there was a heavy crop of apothecia, in the second autumn there was a small crop, and in the third there were still a few apothecia. Such physiological heterogeneity amongst populations of sclerotia complicates the agricultural problem of eliminating them by any cultivation or other method. Other things being equal, the greater the physiological heterogeneity in a population of sclerotia, the longer is the potential survival of the fungus. This physiological heterogeneity in *S. trifoliorum*, which finds an explanation in the fact that this species is constituted of a diverse range of biotypes (see section on 'co-inoculations' below), is probably an important factor in the persistence of the parasite from one season to another.

(*b*) *Single-spore lines*

Homothallism and heterothallism in the genus *Sclerotinia* has been the subject of much recent investigation, and Buller (1941) states that indications point to the fact that '*Sclerotinia* as a genus is heterothallic rather than homothallic'. Whetzel (1945), however, has emphasized that the genus *Sclerotinia*, as formerly constituted, embraces a heterogeneous collection of genera, and has proposed that it should be split up into a number of separate genera, including *Botryotinia* (e.g. *B. fuckeliana*), *Monilinia* (e.g. *M. laxa*), *Sclerotinia* (e.g. *S. sclerotiorum*), *Stromatinia* (e.g. *S. gladioli*), and *Seaverinia* (e.g. *S. geranii*), etc. On the basis of his classification, Whetzel remarks that 'homothallism seems to prevail in *Sclerotinia*, while in *Botryotinia* heterothallism seems the rule'.

Sclerotinia trifoliorum Erikss. is included in Whetzel's genus *Sclerotinia*, and has been shown to be homothallic by Henson (1935) and Keay (1939). Although Keay also attempted to demonstrate homothallism in *S. trifoliorum* var. *fabae*, no apothecia were obtained from single-spore strains. All the Sclerotiniae that have been shown to be homothallic produce microconidia in monospore cultures. No function has been assigned to microconidia in these homothallic species, but, if they are spermatizing agents, the species are self-fertile. *S. trifoliorum* var. *fabae* is closely allied to *S. trifoliorum*, and also produces microconidia in monospore cultures; further the writer has observed apothecial stipes to develop in test-tube cultures of such isolates.

Hence Keay's failure to obtain apothecia of *S. trifoliorum* var. *fabae* from single-spore strains was probably due to unfavourable conditions rather than due to the fungus being heterothallic.

To demonstrate homothallism in *S. trifoliorum* var. *fabae*, sclerotia from eighteen single-spore lines were buried in damp sterile sand. Eight of these single-spore lines represented the eight ascospores from a single ascus, the remaining ten were isolated from several apothecia. The ten random single-spore lines all produced normal apothecia, as also did seven of the eight lines isolated from a single ascus. The eighth line of this latter series produced only one apothecial stipe which aborted before expanding into an apothecium. As the sclerotia in these pots were buried at a depth of $\frac{1}{2}$–$\frac{3}{4}$ in., the possibility that spermatization was effected by insects carrying microconidia of the appropriate sex is most remote. These results may be taken to establish for the first time the homothallic condition in *S. trifoliorum* var. *fabae*. Additional experiments with single-spore strains of *S. trifoliorum* have verified that this fungus is also homothallic.

(2) *Morphology of the apothecia*

(*a*) *Diameter of the disk*

The size of an apothecial disk is of little value by itself as a character for distinguishing species of *Sclerotinia*, for the size range of any one species may overlap considerably with that of another. In addition, apothecial size may be profoundly affected by the previous nutrition of the sclerotium (Keay, 1939) and, when mature, by the prevailing atmospheric humidity. However, in spite of the above sources of variation, specific size differences occur. There can be no doubt, for instance, that in nature the apothecia of *S. serica* Keay are much smaller than those of *S. trifoliorum* or *S. sclerotiorum*.

In an investigation of the relationship of *S. trifoliorum* to the *Sclerotinia* on beans, it seemed worth while to compare the frequency distribution of apothecia of these two fungi according to size. Sclerotia of both fungi that had been cultured on the same medium (i.e. 2·5 % malt agar) were buried in March 1946 and they produced apothecia the following October. Measurements of the disk diameters were made on the same day in October for all the apothecia that were available, irrespective of the state of their maturity. This failure to take into account the state of their maturity (which, in any case, could have been assessed only by an arbitrary standard) is justifiable, since the apothecia of both fungi appear at the same time of year and are similar in duration. Further, it does not affect the object of the comparison, which is to see if apothecial size provides any evidence as to whether *S. trifoliorum* and the *Sclerotinia* on beans belong to the same or different populations. In all, 150 apothecia of *S. trifoliorum* (one hundred from red clover, thirty-four from sainfoin and sixteen from trefoil) and forty apothecia of the *Sclerotinia* on beans were measured. The results showed that apothecial size offers no basis for distinguishing between populations of the two fungi.

(*b*) *Shape of the apothecium*

One of the criteria used by Keay for the comparison of *S. trifoliorum* with the variety *fabae* is the shape of the apothecium. It is stated that in *S. trifoliorum* the apothecial stalk is frequently swollen beneath the disk, gradually increasing in diameter from above soil-level, whereas in var. *fabae* it is stout and of a uniform width; further, the disks of the latter tend to be shallower than those of the normal strains which generally have a deep central depression. It is admitted, however, that it is frequently difficult from a cursory examination to distinguish between them.

From 3 years' observations the writer concludes that the apothecia of both fungi are so variable that their shape has no diagnostic significance. Apothecia corresponding to each of Keay's descriptions can be found amongst populations of apothecia from either beans or clover, and it is considered that these type-descriptions merely represent extremes of a range of shapes common to both fungi. Also there is no obvious indication that either fungus is characterized by a preponderance of apothecia approximating to one shape. If Keay's descriptions are valid, the observed variation would have to be explained on the basis of mixed populations. To cover this possibility spores were measured from four apothecia corresponding to Keay's description for *S. trifoliorum* and four corresponding to her description for var. *fabae*, both selected from among a population of apothecia derived from sclerotia on beans. Spore measurements were also made for a similar eight apothecia produced from sclerotia on clover. This procedure assumes a spore difference between strains of *S. trifoliorum* on clover and those on beans, but evidence is presented later to show that such an assumption is generally true. The frequency distribution of these measurements showed conclusively that apothecial shape is independent of the possible varietal status of the *Sclerotinia* on beans.

(*c*) *Histology of the apothecium*

In the absence of any external difference in size or shape between the apothecia of *S. trifoliorum* and those of the alleged var. *fabae*, a criterion of difference was sought along microscopical lines. Apothecia were examined under a binocular microscope for the presence of hairs or scales on the stipe and on the underside of the disk, a character which Gregory (1942) found useful for characterizing the apothecia of *Sclerotinia polyblastis* Gregory (= *Botrytis polyblastis* Dowson) and *Sclerotinia narcissicola* Gregory (= *Botrytis narcissicola* Kleb.). In both the fungi under consideration, however, the underside of the disk and the aerial part of the stipe are smooth, although the subterranean part of the stipe may bear a few rhizoidal hairs.

In an attempt to detect any histological differences, a large number of microtome sections of mature apothecia were cut and stained. These failed to reveal any tissue differentiation that might be connected with a taxonomic difference. The apothecia of both fungi possess the histological features that are typical of the genus *Sclerotinia* (Pl. 4, fig. 1). In essential points of morphology and histology, therefore, the apothecia of the two fungi would seem to be indistinguishable.

Ascospores

In the preceding section certain morphological characters of the apothecium were considered but rejected as possible criteria for the separation of var. *fabae*. In the present section the more important character of spore size is considered.

The erection of var. *fabae* by Keay (1939) is primarily dependent upon a statistical difference (as measured by Fisher's *t* test) in the difference of the mean lengths of spores from one strain of *Sclerotinia trifoliorum* from clover and three strains of *S. trifoliorum* from beans. This statistical difference is taken to indicate in its own right a consistent difference in spore size between strains on the two hosts.

In any comparative work it is absolutely necessary to know how much the fungi under consideration vary within the species for the character being studied. When a character differs widely within a population (i.e. the population may be composed of a number of smaller populations), the character can only be used for characterization if a sufficient number of samples, to include the range of possible variation, is used in computing the means to be compared. Thus, in the present instance, if there is much variation between spores from separate apothecia of *S. trifoliorum*, it is impossible to draw any valid conclusions on the basis of spore measurements from a few apothecia, however large a number of spores from them are examined. Keay states that 'more than one sample of ascospores of each species were measured'; this tacit assumption of the homogeneity of the populations examined is not justifiable without further investigation. To test the degree of variation between apothecia it is necessary to measure ascospores from widely separated localities, and to analyse the measurements by the method of Analysis of Variance (Fisher, 1936).

It became evident at an early stage in this investigation that spore samples from separate apothecia often differed significantly, and therefore that an examination of a large number of apothecia from many sources was necessary before any variety could be delimited on spore length. The method has therefore been to measure small samples (fifty spores per sample) from a large number of apothecia, rather than vice versa.

(1) *Methods of collection and isolation*

The ascospores of *S. trifoliorum* (and var. *fabae*) are forcibly discharged from the apothecium and the phenomenon of 'puffing', in which thousands of asci discharge almost simultaneously, has been observed on many occasions. Exposure of microscope slides or cover-glasses above a discharging apothecium provides a ready method for collecting samples of ascospores. For most purposes it has been found convenient to support the collecting surface at a height of $\frac{1}{2}$–$\frac{3}{4}$ cm. above the apothecium. No adhesive substance is necessary in the collection of the ascospores, for the spore surfaces at the time of discharge are mucilaginous and cause the spores to adhere to the glass substratum. Ascospores collected on a clean glass slide may be fixed, washed and stained without any noticeable loss in their number. With the collecting surface at $\frac{1}{2}$ cm. or less above the discharging apothecium, the eight spores

from each ascus are typically collected as a single mass, so that many separate groups of eight may be deposited (Pl. 4, fig. 2).

The large number of ascospore samples to be examined in this investigation precluded the measurement of living spores. Thus, the following technique of fixation and staining was adopted so that samples could be kept indefinitely and examined when convenient. The freshly collected spore sample was fixed for 5 min. in formal-acetic-alcohol, washed in distilled water for a further 5 min., and then mounted in cotton blue in lactophenol. The time of fixation was rigidly adhered to so as to standardize as nearly as possible the degree of contraction of the spores in the fixative. Five minutes was chosen as the standard time for fixation because preliminary experiments showed that contraction in the fixative was complete in 4 min.; the extra minute was allowed as a margin of safety. The coefficient of linear contraction for 5 min. immersion in formal-acetic-alcohol was found to be 0·18 for length and 0·25 for breadth. These figures were arrived at by measuring discharged spores in water, and then remeasuring them after fixation and staining. The method was to map the spores measured in the water, and then to remeasure the same spores after treatment. There was also very close agreement with the results obtained by this 'mapping' method when fifty or one hundred spores were measured at random from a sample both before and after treatment. Having derived the coefficient to allow for the contraction in the fixative, the necessary correction factor can readily be introduced into the calculation converting the micrometer eyepiece measurements into terms of microns.

Use has also been made of the forcible ejection of the ascospores for the purpose of establishing single-spore cultures. The method used was to allow a mature apothecium to discharge ascospores on to a layer of agar, 2–3 mm. thick, on a coverglass. Single ascospores were isolated from the deposit so obtained by the 'carrying' procedure of Dickinson's method (Dickinson, 1933). The simple device, described by Dickinson, of using the condenser and mechanical stage of the microscope to obviate the use of a micromanipulator, has proved eminently satisfactory for the isolation of single spores of this species. Dickinson's method of isolation permits any chosen spore to be transferred to the edge of the agar film, and the new position marked out by slits cut in the agar surface with the point of the glass needle. The squares marked on the agar, containing the separated spores, are cut out, examined under the microscope to confirm that in fact only one spore has been isolated, and then transferred with a sterile scalpel to stock culture tubes. As the ascospores of *S. trifoliorum* can be collected in groups of eight, this method was successfully applied in obtaining sets of eight ascospore lines from individual asci. The eight ascospores of a group could usually be separated from one another with ease, but a certain amount of difficulty was encountered when strands of the residual epiplasm (which is discharged along with the spores) caused two or more spores to stick tenaciously together. Damage by pressure from the glass needle during separation probably accounted for the failure to germinate of one or more spores in several of

the sets of eight that were isolated. The uses to which the random monospore lines and the sets of eight were put are described in the text.

(2) *Ascospore measurements of strains from clover and beans*

It has been suggested above that the variety *fabae* Keay of *S. trifoliorum* Erikss. was erected on insufficient evidence. The validity of according varietal rank to the bean strains of *S. trifoliorum* can only be determined by ascospore measurements of clover and bean strains from widely separated localities. Ascospore measurements have been made in the present study to provide the necessary evidence for deciding

TABLE 2. *Spore lengths (units of micrometer eyepiece) of strains of* Sclerotinia trifoliorum *from clover and beans (totals of fifty spores per apothecium)*

County	Locality	Apothecium 1	2	3	4	5	Total*
		Clover collections					
Cambs	Cambridge	445	443	442·5	439·5	446	316
	Landbeach	436·5	435·5	463	450	438	323
	Thriplow	412·5	452·5	422	419·5	404	210·5
Essex	Danbury	440	453	420·5	385·5	414	213
	Gt. Bromley	466·5	440	419·5	451	437	314
	Southminster	467	459	420	429	436	311
	Stapleford Abbotts	439	413	429	417	447	245
Herts	Harpenden	425·5	426·5	446	420	425·5	243·5
	Hertford	419	422·5	424	414·5	429	209
Norfolk	Heacham	436	415	416	391·5	427·5	186
	Wighton	439	433	428	418·5	426	244·5
Suffolk	Occold	434	418	413	432	448·5	245·5
Northants	Peterborough	410·5	432	417	429	416	204·5
Somerset	Watchet	458·5	431	437	443	417	286·5
Glos.	Acton Turville	447	420	439	431	419	256
						Total	3808
		Bean collections					
Cambs	Cambridge	508	525	510	516	485	644
	Comberton	510	489·5	498	484·5	510·5	592·5
	Coton	536	534·5	508·5	484	536	699
	Croydon	521·5	508	498	541	522·5	691
	Landbeach	394·5	405	417·5	416	417·5	150·5
	Longstanton	516	516·5	541	495·5	514	683
	Swavesey	550·5	505·5	515·5	522·5	495·5	689·5
Essex	Gt. Bentley	479·5	509·5	511·5	485·5	497·5	583·5
	Pattiswick	477	528·5	485	488·5	501·5	580·5
Hunts	Hartford	510	489·5	508·5	495·5	495	598·5
Norfolk	Shelton	426·5	417·5	420	427·5	418·5	210
Suffolk	Depden	521·5	516	570	493	581	781·5
	Kirtling	536	489	484	517	528·5	654·5
	Saxmundham	506·5	497	474	526·5	481	585
	Wickham Skeith	525	518	516·5	501·5	510	671
Northants	Hartwell	479·5	528·5	568	515	481	672
						Total	9486

* This column is coded by subtracting 1900 from each total (i.e. 380 from the figure for each of the five apothecia).

whether the name var. *fabae* should be retained or whether the variety should be reduced to a synonym. For this purpose diseased clover plants were collected from fifteen centres distributed in eight counties in the east and west of England, and diseased bean plants from sixteen centres distributed in six counties in the east of England. (N.B. *S. trifoliorum* is only very rarely found on beans in the west of England.) The actual centres are recorded in Table 2. Naturally occurring sclerotia, picked off from the diseased plants, were used for all centres except Watchet and Acton Turville, where sclerotia produced in culture had to be substituted. Spores from five apothecia derived from five separate sclerotia were measured for each centre. Although both length and breadth were measured, attention is focused on spore length because the differences between spores are greatest in this dimension.

Table 2 records the totals of the lengths of fifty spores for each of 155 apothecia, comprising the fifteen centres of *S. trifoliorum* from clover and the sixteen centres from beans ($155 = 5(15 + 16)$). It is desired to use these data to ascertain: (i) whether the variation on each host is consistent with a homogeneous population, and (ii) whether the isolations from clover and those from beans represent different fungi or merely separate collections of the same fungus.

The variation in spore length on each host is analysed in Tables 3 and 4. In calculating the respective sums of squares the data are coded by subtracting 380 from each total of fifty spores, i.e. $5 \times 380 = 1900$ from the total of each centre.

TABLE 3. *Analysis of variance of the spore-length totals of the clover samples*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between centres	14	5,908·0	422·0
Error	60	12,784·6	213·1
Total	74	18,692·6	—

In Table 3 the calculated value of F (variance ratio) for the between-centres/error comparison is 1·98, which is significant at the 5 % level but not at the 1 % level. There is thus some evidence of variation between localities additional to that at one locality. The same conclusion is reached by comparing the variance estimates, each with 4 degrees of freedom, calculated from the five apothecia totals, by Bartlett's test for homogeneity of variance. The fact that the fifteen clover centres seem to show some degree of variation with locality is, however, not inconsistent with separate collections of the same fungus from widely separated localities.

TABLE 4. *Analysis of variance of the spore-length totals of the bean samples*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between centres	15	86,947·1	5,796·5*
Error	64	25,984·8	406·0
Total	79	112,931·9	—

* Variance which is significantly greater than the error variance at the 1 % point as tested by the F test.

In Table 4 the calculated value of F for the between centres/error comparison is 14·28 which compares with a theoretical value at the 1 % point of 2·34. This means that the bean centres differ widely among themselves, and it is necessary to compare the treatment totals from which the between-centres variance was calculated. The required totals, arranged in order of increasing size, are given in Table 5.

Table 5. *Totals (arranged in order of increasing size) of the spore-length measurements for the sixteen collections of* S. trifoliorum *from beans*

Total*	Collection
150·5	Landbeach, Cambs
210·0	Shelton, Norfolk
580·5	Pattiswick, Essex
583·5	Gt. Bentley, Essex
585·0	Saxmundham, Suffolk
592·5	Comberton, Cambs
598·5	Hartford, Hunts
644·0	Cambridge, Cambs
654·5	Kirtling, Suffolk
671·0	Wickham Skeith, Suffolk
672·0	Hartwell, Northants
683·0	Longstanton, Cambs
689·5	Swavesey, Cambs
691·0	Croydon, Cambs
699·0	Coton, Cambs
781·5	Depden, Suffolk

* Data coded by subtracting 380 from each of the five totals for the five apothecia in each collection.

Each of the figures in Table 5 represents the total of five apothecia so that the standard error of the difference between any pair is $\sqrt{(406{\cdot}0 \times 5 \times 2)} = 63{\cdot}72$. The number of degrees of freedom of the error variance is 64, and the value of t for a probability of 0·01 is approximately 2·65. A difference between these totals greater than $63{\cdot}72 \times 2{\cdot}65 = 169$ is therefore significant. From Table 5 it will be seen that the Landbeach and Shelton totals are clearly distinct from those of the other centres; it should be noted, however, that they approximate to the values applicable to the clover centres. The probability of the Landbeach and Shelton samples being drawn from a population which includes the other fourteen centres is very much less than 0·001. Of the remaining fourteen bean centres, Depden (781·5) is the only one with a total significantly greater than that of Pattiswick, which has the smallest total (580·5). Depden is not significantly different, however, from Cambridge (644·0) or the other seven centres with totals greater than that of Cambridge. The bean centres, other than Landbeach and Shelton, may thus be regarded as one series. Although there is a sharp break in the continuity of the totals between Coton (699·0) and Depden (781·5), this discontinuity would probably be bridged in the event of more centres being measured. The conclusion that the bean centres other than Landbeach and Shelton constitute one series can also be shown by drawing up a revised analysis of variance table (Table 6) for the bean samples in which these two centres are excluded. The exclusion of these two centres is justifiable on grounds of spore size

alone, but is further substantiated by the fact that isolates from them are subsequently shown to be culturally distinct from the other bean isolates and identical with the clover isolates. The calculated value of F for the between-centres/error comparison in Table 6 is 1·47, which is not significant even at the 5 % level. Thus there is no definite evidence of heterogeneity in the remaining fourteen bean centres. Bartlett's test also gives a non-significant result.

TABLE 6. *Revised analysis of variance table for the bean samples, with the Landbeach and Shelton samples excluded*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between centres	13	8,748·4	672·9
Error	56	25,582·0	456·7
Total	69	34,330·4	—

Having tested for heterogeneity on the two hosts, the next step is to compare the clover samples considered as one unit with the bean samples considered as another unit. In this comparison (Table 7) the Landbeach and Shelton samples are omitted altogether, although (as will be evident later) they could have been included in the clover series. The calculated value of F for the between-hosts/error comparison in Table 7 is 621·6, which is very highly significant, in spite of the fact that the clover

TABLE 7. *Analysis of variance table for the comparison of spore length with respect to host*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between hosts	1	230,114·5	230,114·5
Error	143	52,925·0	370·1
Total	144	283,039·5	—

centres seem to show some variation with locality and the bean centres may possibly also be heterogeneous. Since this result is based on isolates from widely separated localities, it may be concluded that there is a definite difference in spore size between the strains of *S. trifoliorum* on clover and those on beans. The existence of a distinct var. *fabae* is thus vindicated. As considerably more data have been accumulated than in the original work by Keay, the spore sizes of *S. trifoliorum* and *S. trifoliorum* var. *fabae* may be fixed with greater precision. In converting the micrometer eyepiece measurements into microns, the correction factor to allow for the contraction in the fixative has been introduced. On the basis of the seventy-five apothecia of *S. trifoliorum* from clover and the seventy (80 – 10) apothecia of *S. trifoliorum* var. *fabae* from beans, the spore sizes of the two fungi are defined in Table 8. The values obtained agree extremely closely with the average dimensions given by Keay.

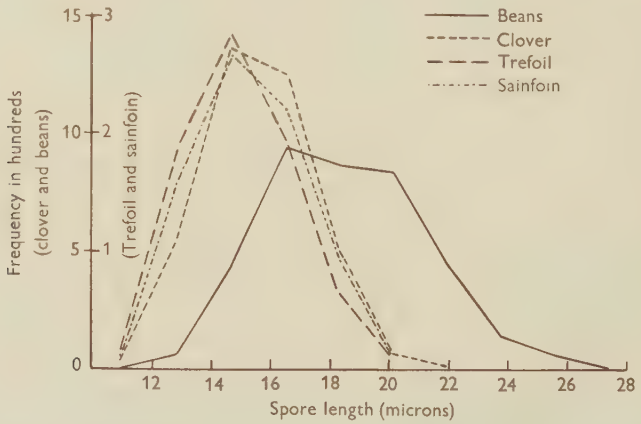
TABLE 8. *Dimensions of ascospores of* Sclerotinia trifoliorum *Erikss. and* S. trifoliorum *Erikss. var.* fabae *Keay*

Name of fungus	Length (μ) Mean	Length (μ) S.D.	Breadth (μ) Mean	Breadth (μ) S.D.
Sclerotinia trifoliorum	15·77	1·71	8·07	1·07
Sclerotinia trifoliorum var. *fabae*	18·66	2·63	9·52	1·64
(Keay's figures)				
Sclerotinia trifoliorum	15·23	1·94	8·67*	1·27
Sclerotinia trifoliorum var. *fabae*	19·02	2·44	9·97	1·72

* This figure was published in error as 8·17.

(3) *Ascospore measurements of strains from trefoil and sainfoin*

Although there was no reason to suspect that isolates of *S. trifoliorum* on leguminous hosts other than beans were in any way different from the normal strains as found on clover, the confirmation of a distinct var. *fabae* necessitated that spore measurements should be made to substantiate such an assumption.



Text-fig. 1. Frequency distribution, according to length, of spores of *S. trifoliorum* from clover (3750 spores), trefoil (750 spores) and sainfoin (750 spores), and spores of *S. trifoliorum* var. *fabae* from beans (figures based on 3500 spores but multiplied by 75/70 so as to be applicable to a total of 3750 spores).

Spores were measured from fifteen apothecia derived from sclerotia on trefoil, and fifteen from sclerotia on sainfoin. In both cases the fifteen apothecia represented three separate centres (five apothecia per centre). Fifty spores were measured from each apothecium, thus making 750 spores from each host. The results are presented graphically in Text-fig. 1. The frequency distribution of the lengths of the spores from the seventy-five apothecia of *S. trifoliorum* from clover and the seventy apothecia

of var. *fabae* from beans, which were measured in the previous section, are included for comparison. The var. *fabae* figures (based on seventy apothecia) are modified so as to conform with those of *S. trifoliorum* from clover, i.e. multiplied by 75/70 so as to appear as if based on seventy-five apothecia instead of seventy.

The frequency distribution curves in Text-fig. 1 indicate no difference in spore length between the strains on trefoil and sainfoin and those on clover. This agrees with their cultural behaviour (see section on 'co-inoculations' below). It may be concluded therefore that there are no varieties of *S. trifoliorum* peculiar to trefoil and sainfoin in the same way as for beans.

Cultural studies

(1) *Growth rates*

Data on the rate of lateral extension of mycelium are not adequate for the delimitation of species, for two strains of the same morphological species may show differences in respect of this criterion greater than those between separate species. Such data, however, may be informative in cases where two organisms are suspected of being different on other grounds.

In her study of certain species of the genus *Sclerotinia*, Keay (1939) records a difference in the rate of lateral extension of mycelium of *S. trifoliorum* and *S. trifoliorum* var. *fabae*. Keay carried out two experiments with two strains of *S. trifoliorum* and three experiments with two strains of *S. trifoliorum* var. *fabae*. The differences in the results of the two experiments with *S. trifoliorum* were only slight and did not overlap with those of the three experiments with *S. trifoliorum* var. *fabae*. Keay does not suggest that this observation has any taxonomic importance, but, if it is consistent, it may be useful in the present investigation for supplementing on a cultural basis the conclusions already derived from spore measurements.

With this object alone in view, four single-spore isolates of *S. trifoliorum* (nos. 1–2 from clover, no. 3 from sainfoin, and no. 4 from trefoil) and four single-spore isolates of the *Sclerotinia* on *Vicia faba* (nos. 5–6 agreeing in spore size with *S. trifoliorium* var. *fabae*, and nos. 7–8 agreeing with *S. trifoliorum*) were grown on 5 % malt-extract agar at 15° C. It was thought possible that the extension rates of isolates nos. 7–8 might approximate more closely to those of nos. 1–4 than to those of nos. 5–6. To standardize as nearly as possible the previous histories of the different isolates, they were inoculated before the experiment on to their appropriate host plants and then re-isolated on to Dox's agar. While the cultures were still young a second transfer was made to Dox's agar and these cultures were used to inoculate the 5 % malt-extract 'inoculation plates'. When approximately half of the medium of the inoculation plates had been covered, the inocula were cut with a borer of diameter 2 mm. from just within the growing edge and placed, mycelial surface downwards, in the centre of the 'experimental plates'. The lower halves of the experimental plates had an internal diameter of 9·1 cm. and contained 20 c.c. of

5 % malt-extract agar, which was poured from test-tubes into which it had been measured before sterilization. Four plates were used for each strain.

The colonies were symmetrical and were measured along two diameters at right angles. The average of these two values represented the size of any one colony, and the group mean was the average of the four separate cultures. The measurements were made at the experimental temperature. Zero time was taken as the time when the cultures were placed in the 15° C. chamber, immediately after inoculation.

Plotting the data at the various observation points on a size-time graph showed that the phase of constant lateral spread was quickly reached, i.e. when the colonies were only 20 mm. in diameter. In view of the slight differences between the isolates in the time taken to make any measurable growth, the author considers that, for such fast-growing fungi as these, the rate of *constant* lateral spread gives a better measure of comparison than one which registers from zero time. In comparing the eight isolates, therefore, the initial phase of growth has been ignored, and the time in hours which each took to increase from a diameter of 20–80 mm. has been used as the basis of comparison. The results are given in Table 9. Keay's data (unpublished) for the lateral spread on the same medium at 15° C. of one strain of *S. trifoliorum* and two strains of *S. trifoliorum* var. *fabae* have been modified to conform with the above definition, and are included in Table 9 for comparison.

Table 9 shows that different isolates of *S. trifoliorum* vary widely as regards their rate of lateral spread of mycelium, but that they cannot be separated on this basis into two cultural groups corresponding with *S. trifoliorum* and *S. trifoliorum* var. *fabae*. It seems safe, therefore, to conclude that rate of lateral spread of mycelium does not parallel the separation on spore size of the variety *fabae* from typical *S. trifoliorum*.

TABLE 9. *Time, in hours, taken by isolates of* Sclerotina trifoliorum *and* S. trifoliorum *var.* fabae *to increase in diameter from* 20 *to* 80 *mm. at* 15° C.

Isolate	Spore size in agreement with	Host from which isolated	Time (hr.) to increase from 20 to 80 mm.
1	*S. trifoliorum*	Clover	80·9
2	*S. trifoliorum*	Clover	76·1
3	*S. trifoliorum*	Sainfoin	70·8
4	*S. trifoliorum*	Trefoil	69·5
5	*S. trifoliorum* var. *fabae*	Beans	70·5
6	*S. trifoliorum* var. *fabae*	Beans	73·5
7	*S. trifoliorum*	Beans	93·5
8	*S. trifoliorum*	Beans	91·9
Keay's data:			
Clover I	*S. trifoliorum*	Clover	61·2
Bean I and II	*S. trifoliorum* var. *fabae*	Beans	59·5*

* Average of three experiments.

(2) *Co-inoculations*

Records in the literature repeatedly state that different isolates of *S. trifoliorum* vary considerably in respect to such characters as growth rate on artificial media and general cultural habit (number and size of sclerotia, etc.); similar differences have been found among the isolates examined by the author. Some workers (Nilsson-Leissner & Sylven, 1929; Pape, 1937) have considered that these differences point to the existence, in this species, of physiological specialization comparable in some respects to that found in rusts, smuts and certain ascomycetes. Keay (1939), however, has shown on the basis of mycelial inoculation that, of five strains of *S. trifoliorum* from four different hosts, no one strain was consistently more pathogenic than another. Each strain severely rotted all the members of a selected differential series of five leguminous species. More recently Frandsen (1946) has tested isolates of *S. trifoliorum* from seven species of pasture legumes on a differential series of the same seven species: normal infection occurred in every possible combination. *S. trifoliorum* cannot therefore be divided into varieties peculiar to host in the same way that *Erysiphe graminis*, for instance, is split into its varieties, *poae*, *agropyri*, *bromi*, *hordei*, etc.

It is important, however, especially from the point of view of the plant-breeder, to know if this species consists of a number of more or less well-defined physiologic races. Theoretically this could be determined by testing the relative pathogenicities of a collection of isolates on a differential series consisting of a large number of leguminous hosts. Such a procedure is not feasible in practice owing to the extreme difficulty of defining degrees of virulence for a pathogen such as this, added to which is the complication that the hosts of *S. trifoliorum* are typically cross-pollinated and therefore occur as populations that are genetically heterogeneous.

In order to gain some idea of the taxonomic status of this species, use has been made of an observation by Björling (1942). He noticed that, when two cultures of *S. trifoliorum* are simultaneously inoculated on opposite sides of the same agar plate, the resulting mycelia are often separated by a distinct zone of demarcation. This dividing region consists of two white lines (sometimes merging into one another so as to appear single), which are formed as a result of increased mycelial growth on either side of the line of hyphal contact. Sclerotial formation tends to be concentrated along these lines (Pl. 4, fig. 4). Between this double line microconidia are produced more abundantly than elsewhere on the plate, and they can usually be seen as a faint streak by holding the plate up to the light.

The formation of a 'demarcation line' is not due to any mechanical barrier set up by the presence of opposing hyphae, for two mycelia of the same monospore culture do not react (Pl. 4, fig. 3). Björling has suggested that two mycelia which react to form a demarcation line are of different genotypes and, conversely, those which do not react are of the same genotype. The results of the following experiments show that Björling's hypothesis is inadequate; although it will be assumed that the

formation of a demarcation line implies a genotypic difference, nothing can be said about two mycelia which fail to react—they may or may not be the same genotype.

For the purpose of determining the range of types of *S. trifoliorum* found in this country, twenty different isolates (fifteen from red clover, three from sainfoin, and two from trefoil) were paired in all possible combinations. These isolates were collected from seventeen separate centres, distributed over eight counties of eastern England and two counties in the west of England. They were numbered from 1 to 20. Isolates nos. 1–10 were single-spore lines, isolates nos. 11–20 were hyphal tip cultures derived from sclerotial core isolates. A fortnight to 3 weeks after the pairings were made the cultures were examined and the reactions recorded. The results of these pairings are shown in Table 10, in which a + sign indicates the occurrence of a reaction and a − sign failure to react. In cases of reaction where a line of microconidia could not be seen readily by holding the plate up to the light, the + sign is included in brackets. While this distinction between + and (+) is merely an arbitrary one, that between reaction and non-reaction is absolute. Both + and (+) must therefore imply genetic difference.

TABLE 10. *The results of pairing in all possible combinations twenty isolates of* Sclerotinia trifoliorum. *Nos.* 1–10 *monospore lines, nos.* 11–20 *mycelial lines*

Culture no.	Reaction following co-inoculation with culture nos.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	−	(+)	+	(+)	+	+	+	+	+	+	+	+	(+)	+	(+)	(+)	+	+	(+)	+
2		−	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3			−	+	(+)	+	+	+	(+)	+	+	+	(+)	(+)	(+)	(+)	(+)	(+)	+	+
4				−	(+)	+	+	−	+	+	+	+	(+)	+	(+)	+	(+)	+	+	+
5					−	+	+	+	+	+	+	+	(+)	+	+	+	(+)	+	+	+
6						−	(+)	+	(+)	(+)	+	+	−	+	+	+	(+)	+	(+)	+
7							−	+	+	(+)	(+)	+	(+)	+	(+)	+	(+)	(+)	+	+
8								−	+	+	+	+	(+)	+	+	+	(+)	+	+	(+)
9									−	(+)	(+)	+	(+)	+	+	+	+	(+)	+	+
10										−	(+)	(+)	(+)	+	+	+	+	+	+	(+)
11											−	+	+	(+)	(+)	(+)	+	+	(+)	+
12												−	+	+	+	+	+	+	+	+
13													−	(+)	−	+	+	+	+	+
14														−	+	+	+	+	(+)	+
15															−	+	+	+	+	+
16																−	(+)	+	+	+
17																	−	+	+	−
18																		−	+	+
19																			−	+
20																				−

+ = reaction. (+) = indistinct reaction. − = non-reaction.

Table 10 shows that in no case did an isolate, whether monosporous or mycelial in origin, react with itself but that, with four exceptions, reaction occurred in every other combination. This may be taken to mean that *S. trifoliorum* exists as a large number of biotypes. It would serve no useful purpose to define these biotypes

because it would seem that their number could be extended almost indefinitely as progressively more isolates were tested.

The four cases of non-reaction between different isolates, namely no. 4 against no. 8, no. 6 against no. 13, no. 13 against no. 15 and no. 17 against no. 20, are of particular interest. On Björling's hypothesis isolates nos. 4 and 8 should be the same biotype, and similarly for the other pairs. When the reactions of the separate members of any pair against the remaining eighteen isolates are compared, however, such a conclusion is unlikely. That two isolates which fail to react are not necessarily the same biotype is proved by a consideration of the triangular relationship of isolates nos. 6, 13 and 15. According to Björling, because no. 13 does not react with no. 6, no. 13 equals no. 6. Similarly no. 13 also equals no. 15. Therefore, if Björling is right, no. 6 must equal no. 15. In fact nos. 6 and 15 react together when paired, and therefore cannot be the same biotype.

Since isolate no. 15 was a mycelial isolate it was remotely possible that this result could be explained by some sectoring effect on the part of no. 15. For this reason the three isolates were co-inoculated on the same Petri plate, but the result was the same as that obtained in the original experiment. It is therefore apparent that nothing definite can be deduced about two isolates which fail to react, and therefore that part of Björling's hypothesis which states that two mycelia which fail to react are the same genotype must be discarded.

After the results embodied in Table 10 had been obtained, a similar set of co-inoculations was made with isolates of *S. trifoliorum* from *Vicia faba* to see if a comparable situation held among isolates on this host. The results of pairing fifteen different isolates (nos. 1–10 monosporous, nos. 11–15 mycelial) in all possible combinations are shown in Table 11. In this experiment, in addition to non-reaction and the formation of a demarcation line, a third type of reaction (denoted in Table 11 by an asterisk) was observed. This took the form of a sharp, brown-coloured line (Pl. 4, fig. 5), which was not accompanied by the marked piling up of mycelium on each side of the line of hyphal contact, characteristic of the demarcation line. This brown-coloured line has been arbitrarily designated an 'incompatibility line', to distinguish it from a demarcation line as described previously. So far as could be determined the incompatibility line is formed by the bursting of the hyphal tips when the two strains come into contact. The brown colour is due to the breakdown products of the protoplasm so liberated.

Table 11 shows that, apart from isolates nos. 5 and 11, the inter-reactions of isolates from *Vicia faba* parallel those of *Sclerotinia trifoliorum* proper. In no case did an isolate react with itself but, with two exceptions (no. 1 against no. 14, and no. 2 against no. 9), reaction occurred in every other combination. It may therefore be stated that *S. trifoliorum* var. *fabae* also constitutes a diverse range of biotypes.

Isolates nos. 5 and 11 are characterized by the formation of incompatibility lines when co-inoculated with the other isolates; it is to be noticed, however, that they form a normal demarcation line when they approach one another. Reference to the

TABLE 11. *The results of pairing in all possible combinations fifteen isolates of* Sclerotinia trifoliorum *from* Vicia faba. *Nos.* 1–10 *monospore lines, nos.* 11–15 *mycelial lines*

Culture no.	Reaction following co-inoculation with culture nos.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	−	+	+	+	*	+	+	(+)	+	+	*	+	+	−	+
2		−	+	(+)	*	+	+	+	−	+	*	+	+	+	+
3			−	+	*	+	+	+	+	+	*	+	+	+	+
4				−	*	+	+	+	+	+	*	+	+	+	+
5					—	*	*	*	*	*	+	*	*	*	*
6						−	(+)	+	+	+	*	+	+	+	+
7							−	+	+	+	*	+	+	+	+
8								−	+	+	*	+	+	+	+
9									−	+	*	+	+	+	+
10										−	*	+	+	+	+
11											−	*	*	*	*
12												−	+	+	+
13													−	+	+
14														−	+
15															−

\+ = demarcation line. * = incompatibility line.
(+) = indistinct demarcation line — = no reaction.

spore sizes of the collections from which nos. 5 and 11 were isolated, revealed that they were representative of the two collections of *S. trifoliorum* on beans (namely Landbeach (no. 11) and Shelton (no. 5)) which gave measurements in accordance with the strains on clover. This fact strongly suggests that an incompatibility line is the result of contacting a var. *fabae* strain with a normal strain. To investigate this possibility further, the reactions of the above fifteen bean isolates, together with a culture of one of Keay's original var. *fabae* strains, were tested against ten normal strains of *S. trifoliorum* (nos. 1–6 from clover, nos. 7–8 from trefoil, and nos. 9–10 from sainfoin). The results are given in Table 12.

TABLE 12. *The reactions of sixteen isolates of* Sclerotinia trifoliorum *from* Vicia faba *against ten isolates of* S. trifoliorum *from clover, trefoil and sainfoin*

S. trifoliorum isolate	Reaction following co-inoculation with bean isolate nos.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	*v.f.* (Keay)
1	*	*	*	*	+	*	*	*	*	*	+	*	*	*	*	*
2	*	*	*	*	+	*	*	*	*	*	+	*	*	*	*	*
3	*	*	*	*	+	*	*	*	*	*	+	*	*	*	*	*
4	*	*	*	*	+	*	*	*	*	*	+	*	*	*	*	*
5	*	*	*	*	+	*	*	*	*	*	+	*	*	*	*	*
6	*	*	*	*	+	*	*	*	*	*	+	*	*	*	*	*
7	*	*	*	*	+	*	*	*	*	*	+	*	*	*	*	*
8	*	*	*	*	+	*	*	*	*	*	+	*	*	*	*	*
9	*	*	*	*	+	*	*	*	*	*	+	*	*	*	*	*
10	*	*	*	*	+	*	*	*	*	*	+	*	*	*	*	*

* = incompatibility line. + = demarcation line.

From Table 12 it can be seen that the formation of an incompatibility line is not peculiar to bean isolates nos. 5 and 11, but is the general response to contacting a var. *fabae* strain with a normal strain. The apparently exceptional behaviour of isolates nos. 5 and 11 in Table 11 is therefore to be explained by the fact that these isolates belong to *S. trifoliorum* proper, and not to *S. trifoliorum* var. *fabae* which comprises the other strains considered in the experiment. The exclusion of strains nos. 5 and 11 from the calculation, in an earlier section, to define the spore size of var. *fabae* is thus fully justified.

The formation of an incompatibility line whenever a var. *fabae* strain contacts a normal strain has an obvious application in identification. If it is desired to know whether an isolate of *Sclerotinia* from a specified legume belongs to *S. trifoliorum* or *S. trifoliorum* var. *fabae*, all that is necessary is to test its reaction against a strain of which the identity is already known. This obviates the necessity of measuring a large sample of spores (a large sample is essential on account of their variability) and the inevitable delay, which may be anything from a few weeks to months, in obtaining apothecia. The author used this 'incompatibility line' method for identifying two strains that were not incorporated for spore measurements because their place of origin was unknown. The first strain was isolated by Professor F. T. Brooks from diseased beans, the second was cultured from a sclerotium present among red clover seed. The reaction of Professor Brooks's isolate was interesting because it showed that this isolate belonged to *S. trifoliorum* and not to *S. trifoliorum* var. *fabae*, as might have been expected. This is thus the third case of a normal strain of *S. trifoliorum* having been isolated from beans. The reaction of the second isolate agreed with expectation in conforming to that of *S. trifoliorum*.

(3) *Heterokaryosis*

To account for the great diversity of biotypes of *S. trifoliorum* and *S. trifoliorum* var. *fabae*, as shown by their reaction in culture, it is necessary to demonstrate the operation of a mechanism which is capable of constantly producing new biotypes. Since the vegetative stage of these fungi is haploid, the obvious possibility, in the absence of heterothallism, would seem to be heterokaryosis.

Before this possibility can be considered reference must be made to the cytology of ascus development in *S. trifoliorum*. This subject has been studied by Björling (1942), whose results have been confirmed by Frandsen (1946). In the stalk of the young apothecium ascogenous hyphae are formed at an early stage, without the previous development of special sex organs. In the ascogenous hyphae the nuclei occur in pairs; this initiates the dikaryotic $(n+n)$ phase. The paired nuclei undergo conjugate nuclear division, usually with the formation of a succession of 'clamp connexions'. The ascogenous hyphae often branch in such a way that several asci are formed from the same original hypha. In the terminal (ultimate) cells of the ascogenous hyphae which develop into asci the two nuclei of the pair fuse to form the primary ascus nucleus, which is thus diploid $(2n)$. The primary ascus nucleus

then undergoes three successive divisions to give a linear row of eight nuclei, around each of which a spore is organized. Two further mitotic divisions within the ascospore result in the mature ascospore being quadrinucleate. The ascus is long and narrow, and, as always with asci of this shape, the first two divisions in the ascus are longitudinal while the third is oblique or transverse. There can be no doubt that in *S. trifoliorum* there is only one nuclear fusion and one reduction division, because eight bivalents can be counted with fair certainty in the division of the primary ascus nucleus, and the same number of chromosomes can be observed in the divisions that occur in the hyphae of the germinating ascospore (Frandsen, 1946).

With the above knowledge as the background in which the possibility of heterokaryosis is to be interpreted, there are two observations that can be regarded as circumstantial evidence for the possible occurrence of heterokaryosis:

(*a*) *Anastomosis*

Anastomoses between hyphae from two germinating ascospores were frequently observed in the course of germination tests. If it is assumed that anastomoses between hyphae of different biotypes give rise to heterokaryotic mycelia, then there is the possibility of fusion in the young asci between nuclei of different genotypes and the segregation of new types.

(*b*) *Different spore sizes within an ascus*

It has already been pointed out that the eight ascospores of an ascus are typically ejected together, so that groups of eight may be collected on a glass slide. A frequent but *not* invariable feature of such groups, particularly those of var. *fabae*, is the wide variation in spore size that may exist between the component members of a group. In extreme instances, there would seem to be a more or less clear-cut differentiation into four larger and four smaller spores (Pl. 4, fig. 6). This differentiation could be explained as due to some mechanical or physiological effect consequent upon an axial gradient within the ascus. That this is not so in the present case is shown by the fact that both first division segregation patterns can be observed in undischarged asci, i.e. the four larger spores may be either proximal or distal in the ascus. The occurrence of four larger and four smaller spores would thus seem to rest on a genetical basis. Segregation for spore size provides an obvious manifestation of heterokaryosis, but heterokaryosis may well take place without any such segregation being apparent.

To prove experimentally the occurrence of heterokaryosis it would be necessary to cross single-spore lines and then to test for segregation in the spore progeny by co-inoculating the component members of the asci against the parent types. If, however, segregation of biotypes can be demonstrated in the ascus, independently of a knowledge of the parent types, then clearly this is irrefutable evidence of heterokaryosis in the ascus-initial. Owing to the unsuitability of *S. trifoliorum* for

crossing experiments (only one generation is possible in a year), the latter approach has been the one adopted in this investigation.

If heterokaryosis is taking place to any extent in nature, as is likely from the heterogeneity of *S. trifoliorum*, manifestly it should be possible to detect its occurrence by isolating the eight ascospores of a number of asci derived from sclerotia produced in nature. It was thought unlikely that sclerotia formed on diseased plants in the field would represent mono-ascospore infections. Accordingly the contents of ten asci (two of *S. trifoliorum* and eight of *S. trifoliorum* var. *fabae*) from natural inoculum, and, for comparison, the contents of two asci (of var. *fabae*) from two separate single-spore lines, were isolated by Dickinson's method as described above. This method of isolation gives no information about the position of the ascospores within the ascus, but, since the object of this study is not a genetical one, it enables the required information to be obtained without the risk of damage to the spores incurred by the microdissection of asci. The single ascospore colonies from each ascus were numbered arbitrarily from 1 to 8. Colony no. 1 was then co-inoculated with nos. 2–8, and the development or otherwise of a demarcation line recorded after 2 to 3 weeks. The reactions for any one ascus conformed to one of two patterns, as follows:

	1	2	3	4	5	6	7	8
Pattern A (1)	–	–	–	–	–	–	–	–
Pattern B (1)	–	–	–	–	+	+	+	+

+ = demarcation line. – = no reaction.

In pattern B the identity of nos. 5–8 was shown by a further eight co-inoculations as follows:

	1	2	3	4	5	6	7	8
(5)	+	+	+	+	–	–	–	–

Pattern B can only be explained as the result of a heterokaryon in the ascus-initial; pattern A is probably the result of a homokaryon but, since nothing definite can be deduced about two isolates which fail to react, it cannot be assumed that this is always so.

Of the twelve asci analysed, pattern A was found in the two asci of *S. trifoliorum*, in two out of the eight asci of var. *fabae* from nature, and, as would be expected, in the two asci from the monospore lines of var. *fabae*. Pattern B comprised the remaining six asci of var. *fabae* from nature.

These results establish the occurrence of heterokaryosis (pattern B) in *S. trifoliorum* var. *fabae*, and it is likely that it would also have been demonstrated in *S. trifoliorum* if more asci had been analysed. The results give no indication, however, as to how the heterokaryotic condition arises. In addition to anastomosis, it is possible that the mutation rate is exceptionally high and that mutability and constancy are governed by genetic factors, as has been shown in *Ustilago maydis* (DC.) Corda (= *U. zeae* Unger) by Stakman and his associates (1943). To determine

the extent to which new types are produced in nature as a result of mutation and/or recombinations resulting from interbiotypic hybridization, would involve an extended genetical investigation for which purpose *S. trifoliorum* is not eminently suitable as experimental material.

Discussion

The primary purpose of the above morphological studies was to clarify the status of *Sclerotinia trifoliorum* var. *fabae*. The author has been unable to characterize var. *fabae* by apothecial characters or cultural behaviour (appearance in culture and rate of lateral extension of mycelium). The existence of *S. trifoliorum* var. *fabae*, however, has been confirmed by spore measurements, and the separation is substantiated on the basis of reaction in culture (the formation of an incompatibility line when a normal strain is co-inoculated with a var. *fabae* strain).

Of the seventeen isolates of *S. trifoliorum* from beans examined, fourteen were found to agree in spore size with *S. trifoliorum* var. *fabae* and three with *S. trifoliorum* proper. Var. *fabae* was not found among twenty-two isolates of *S. trifoliorum* from other leguminous hosts (sixteen from red clover, three from trefoil, and three from sainfoin), which all agreed in spore size with *S. trifoliorum* proper. It would thus seem that the *Sclerotinia* disease of beans is usually caused by *S. trifoliorum* var. *fabae* but may be due sometimes to *S. trifoliorum*. For this reason, the Sclerotinia disease of beans may be a contributory factor in clover failure, and to this extent there is some mycological justification for the notion that beans in the rotation may aggravate clover rot. From an agricultural standpoint, therefore, it is unwise to sow beans after clover or clover after beans in a sequence of cropping.

The work described in this paper was carried out under the supervision of Professor F. T. Brooks, C.B.E., F.R.S., to whom I should like to express my sincere thanks for the advice and constructive criticism which he gave throughout the course of the investigation. I am also deeply indebted to Dr W. A. R. Dillon Weston, who suggested the problem to me and who provided me with the facilities for collecting much of the diseased material. Thanks are also due to Dr E. J. Maskell and Dr D. G. Catcheside for discussing the presentation of some of the experimental work, to Mr F. J. Anscombe for checking the statistical data, and to Mr F. T. N. Elborn for taking the photographs.

References

Amos, A. (1918). The difficulties of growing red clover. *J. R. agric. Soc.* **79**, 68.

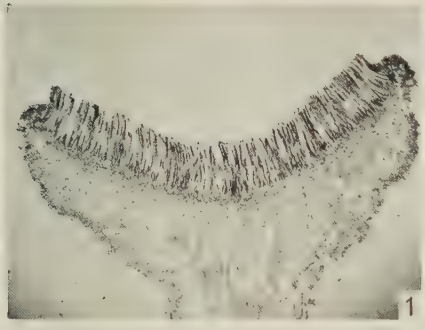
Björling, K. (1942). Undersökningar rörande Klöverrötan, II. Studier av Utvecklingshistoria och Variation hos *Sclerotinia trifoliorum*. *Medd. Växtskyddsanst. Stockh.* **37**, 1.

Buller, A. H. R. (1941). The diploid cell and the diploidisation process in plants and animals, with special reference to the higher fungi. I. *Bot. Rev.* **7**, 335.

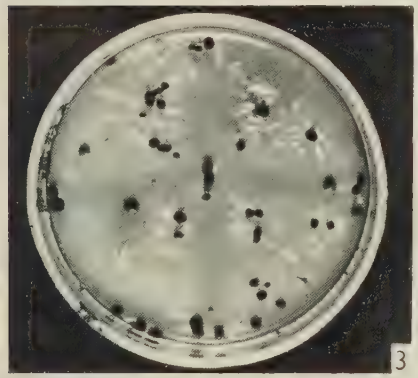
Dickinson, S. (1933). The technique of isolation in microbiology. *Phytopathology*, **23**, 357.

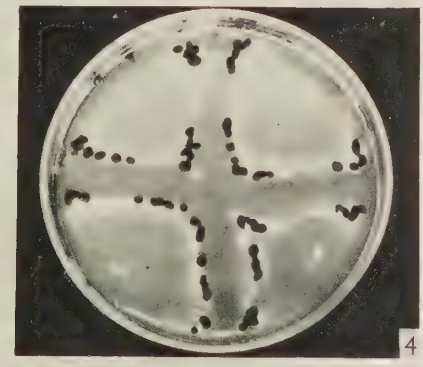
Dillon Weston, W. A. R., Loveless, A. R. & Taylor, R. E. (1946). Clover rot. *J. agric. Sci.* **36**, 18.

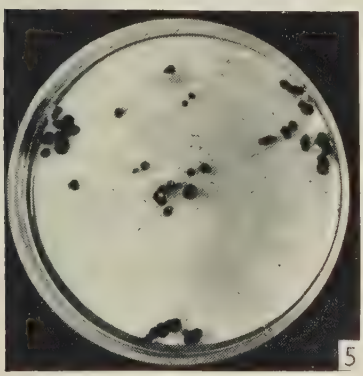
Fisher, R. A. (1936). *Statistical methods for research workers*. Edinburgh: Oliver and Boyd.


1


2


3


4


5


6

Frandsen, K. J. (1946). Studier over *Sclerotinia trifoliorum*. Copenhagen: *Det Danske Forlag*.
Gregory, P. H. (1942). Studies on *Sclerotinia* and *Botrytis*. I. *Trans. Brit. mycol. Soc.* **25**, 26.
Henson, L. (1935). Apothecium production in *S. trifoliorum* and *S. sclerotiorum*. Abst. in *Phytopathology*, **25**, 19.
Keay, M. A. (1939). A study of certain species of the genus *Sclerotinia*. *Ann. appl. Biol.* **26**, 227.
Moore, W. C. (1948). Diseases of crop plants, 1943–1946. *Bull. Minist. Agric.* no. 139. London: H.M.S.O.
Nilsson-Leissner, G. & Sylven, N. (1929). Studier över Klöverrötan (*S. trifoliorum*). *Sverig. Utsädesfören. Tidskr.* **39**, 130. (Cited in *Rev. appl. Mycol.* **8**, 793.)
Pape, H. (1937). Beiträge zur Biologie und Bekämpfung des Kleekrebs (*S. trifoliorum*). *Arb. biol. Abt.* (*Anst.-Reichsanst.*), *Berl.* **22**, 159.
Stakman, E. C., Kernkamp, M. F., King, T. H. & Martin, W. J. (1943). Genetic factors for mutability and mutant characters in *Ustilago zeae*. *Amer. J. Bot.* **30**, 37.
Whetzel, H. H. (1945). A synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate Discomycetes. *Mycologia*, **37**, 648.

EXPLANATION OF PLATE 4

Fig. 1. Longitudinal section of an apothecium of *S. trifoliorum* stained with safranin and light green.
Fig. 2. Two groups of eight ascospores from individual asci, collected on a glass slide after exposure above an actively discharging apothecium.
Fig. 3. Co-inoculation of four isolates from the same single-spore culture: no reaction.
Fig. 4. Co-inoculation of four isolates from four different single-spore cultures: formation of demarcation lines.
Fig. 5. Incompatibility line formed between isolates of *S. trifoliorum* and *S. trifoliorum* var. *fabae*.
Fig. 6. Group of eight ascospores of *S. trifoliorum* var. *fabae*, showing segregation for spore size.

(*Received* 29 *June* 1950)

THE EFFECT OF INFECTION WITH BEET YELLOWS AND BEET MOSAIC VIRUSES ON THE CARBOHYDRATE CONTENT OF SUGAR-BEET LEAVES, AND ON TRANSLOCATION

By M. A. WATSON and D. J. WATSON

Rothamsted Experimental Station, Harpenden, Herts

(With Plate 5)

The loss of total carbohydrate (sugars and starch) per cent of residual dry matter (dry matter less total carbohydrate) during a period of darkness from leaves of sugar-beet plants infected with yellows virus was as great as that from the leaves of healthy plants. The conclusion of previous workers, based on the results of the Sachs iodine test for starch and the occurrence of 'phloem gummosis' in infected plants, that starch accumulates in infected leaves because translocation is prevented by blockage of the sieve-tubes, is therefore incorrect.

Older leaves of infected plants had a higher content of reducing sugars and sucrose, and usually but not invariably of starch, both at the beginning and end of the dark period, than comparable leaves of healthy plants. By far the greater part of the increase was in reducing sugars. In leaves taken in late September from infected plants growing in the field, 20 % or more of the total dry matter was present as reducing sugars. The reducing sugars in both healthy and yellows-infected leaves were shown by paper chromatography to be glucose and fructose in approximately equal amounts.

Accumulation of carbohydrate in infected leaves is probably not a passive consequence of differences in carbohydrate production, distribution and utilization, but is attributable to changes in the physiology of the cells of the leaf.

The carbohydrate content of sugar-beet leaves was little affected by infection with beet mosaic virus.

Yellows-infected leaves had a lower water content per cent of fresh weight than healthy leaves. This was accounted for by the higher carbohydrate content of infected leaves, for the ratio of water:residual dry matter was not affected by infection or was slightly reduced. This implies that hydration was independent of carbohydrate content.

Introduction

When 'Jaunisse' or 'Yellows' of sugar beet was first identified as a virus disease by Quanjer (1934), he described as a diagnostic character a condition of the phloem which he called 'phloem gummosis'. Some of the sieve-tubes in the phloem of the veins and petiole of the leaf contained bright yellow deposits which appeared to block them completely. Quanjer stated that he also found this condition in nine out of twenty-six healthy* plants, though it was more common and extensive in plants with yellows. Roland (1936) found gummosis throughout the phloem of infected

* Throughout this paper 'healthy' is used to signify 'not infected with virus'.

plants, in green leaves, except the very youngest, as well as in yellowed leaves. He concluded that the healthy plants examined by Quanjer were really infected but had not yet shown symptoms, and stated that in his experience gummosis did not occur in healthy plants. Klinkenberg (1945) agreed that gummosis is restricted to infected plants, but maintained that it does not occur until after the appearance of visible leaf symptoms.

When the disease was suspected in Britain, attempts were made to base identification on the presence of phloem gummosis, but many yellowed plants did not show the condition and some apparently healthy plants did. These conflicting results suggest that phloem gummosis is not a specific symptom of yellows infection, but occurs in uninfected plants, possibly associated with senescence or with other diseases, though less frequently than in infected plants.

Quanjer observed that starch is retained in the yellowed leaves of infected plants after periods in darkness long enough to clear it from corresponding leaves of healthy plants. This has been confirmed by several workers. Leaves were taken in the early morning from healthy and infected plants growing in the field, or from plants kept for a period in darkness in the laboratory, and tested for starch by the Sachs test (decolorization with alcohol, followed by treatment with iodine). Infected leaves gave a strong starch reaction, while healthy leaves did not. Combination of the two observations, namely, the occurrence of phloem gummosis and the retention of starch in infected leaves, led to the conclusion that the starch is retained because gummosis blocks the sieve-tubes and prevents translocation.

An alternative hypothesis, that starch might accumulate through a reduction in respiration, was tested by van Riemsdijk (1935). He found that leaves infected with yellows virus have a higher respiration rate than healthy leaves, and concluded that the only explanation for the high starch content was inhibition of translocation.

According to Klinkenberg (1945), carbohydrates accumulate in the leaves of plants infected with yellows before phloem gummosis is apparent. She states that 'gummosis only presents itself when the transport of carbohydrates has already been hindered. This shows that gummosis is not the first and only cause of the impediment of the transport of carbohydrates, but has to be considered as a secondary phenomenon and as a result from the diseased condition of the phloem'.

Clinch & Loughnane (1948) took a similar view. After describing the necrosis of the sieve-tubes, and the hypertrophy, hyperplasia and subsequent collapse of the parenchyma adjoining the phloem associated with 'etch' symptoms in leaves of recently infected plants, they concluded that 'In sugar beet affected by etch yellows, the above mentioned pathological changes...are ephemeral. Their development, however, shows that a disturbance in the phloem is one of the earliest effects of the virus. Considering the nature of the subsequent symptoms of the disease, which Quanjer and van Riemsdijk have shown to be due to inhibited translocation, it seems probable that this phloem disturbance exists permanently in the affected plants, even though further visible changes in the phloem tissues, such as gummosis,

may not take place until after the development of yellowing in the lower leaves.'

Thus, it seems to be generally accepted by workers on beet yellows virus that carbohydrate accumulates in infected leaves because translocation is inhibited by mechanical blocking of the phloem by gummosis or by some invisible change which precedes it.

The object of the present work was to test this hypothesis by making direct measurements to compare the rates of translocation of carbohydrate from healthy and from yellows-infected leaves.

Material and methods

The amount of carbohydrate translocated during a period of darkness was measured by determining the sugar and starch content of comparable samples of leaf tissue taken at the beginning and end of the period. Respiratory loss was assumed to be small compared with loss by translocation. To reduce the sampling variation, the initial and final samples were taken from similar positions in opposite halves of the same leaves. Each sample was made up of disks cut with a punch from similar leaves on a number of plants, so that the samples consisted of equal areas of leaf surface at the time they were taken.

To determine the absolute change in carbohydrate content of the leaves, it is necessary to refer carbohydrate content to a basis that remains constant during the experimental period. Total dry matter obviously does not satisfy this requirement, for the leaves lost dry matter during the dark period. Nor is the difference between the initial and final weights of carbohydrate per sample an accurate measure of the absolute change in carbohydrate content, for although the samples consisted of equal areas of leaf surface at the time of sampling, the initial area of the samples taken at the end of the period must have been less than the area of the samples taken at the beginning, because increase in water content during the period must have caused the leaves to expand.

It is reasonable to assume that loss of carbohydrate was the major cause of change in dry weight and that the change in other dry matter constituents was small and negligible. It follows, as Mason & Maskell (1928) have pointed out, that residual dry matter (dry matter less total carbohydrate) provides an approximately constant basis of reference. The amount of carbohydrate translocated has therefore been expressed as the change in carbohydrate per cent of residual dry matter (R.D.M.). Total carbohydrate is taken as the sum of sugars and starch; this differs slightly from total carbohydrate as defined by Mason and Maskell, for they estimated reserve carbohydrates by acid hydrolysis and therefore probably included other polysaccharides in addition to starch.

As healthy and yellows-infected leaves differ greatly in carbohydrate content, comparisons between them in respect of carbohydrate changes should be made on a basis independent of carbohydrate content, and this is another reason for expressing carbohydrate content in terms of R.D.M.

Experiment 1

The plants used were grown in a field experiment laid down on the Rothamsted farm in 1946 for an investigation of the effect of virus infection on the growth of sugar beet, the results of which will be published in a later paper. The treatments were:

(*a*) Healthy plants (H) *v.* plants infected with beet yellows virus (Y) *v.* plants infected with beet mosaic virus (M).

(*b*) No nitrogenous fertilizer (no N) *v.* 3 cwt. sulphate of ammonia per acre (N).

The six combinations of (*a*) and (*b*) were replicated in two randomized blocks. All the plots received $3\frac{1}{2}$ cwt. superphosphate, 1 cwt. muriate of potash and 2 cwt. agricultural salt per acre; these and the nitrogen dressings were applied before sowing. Kleinwanzleben E seed was sown on 15 April.

The plants of treatment M were infected with mosaic virus on 2–3 July by rubbing the leaves with infective sap. Those of treatment Y were infected at the same time with yellows virus by placing infective aphids on them; the aphids were killed by nicotine fumigation on 5 July.

Samples of leaf lamina were taken at sunset (17.00–18.30 hr. G.M.T.) on 24 September, and soon after sunrise (06.00–07.30 hr.) on 25 September; these are subsequently referred to as evening and morning samples and are given the symbols A and B respectively in the tables. Five pairs of adjacent plants were selected on each plot. The selection was made at random, except that occasional healthy plants on infected plots, infected plants on healthy plots, and plants suffering from other diseases such as downy mildew, were rejected. A disk of area 8·4 sq.cm. was cut with a circular punch from the 15th, 16th, 20th and 21st leaf of each plant counting from the youngest leaf longer than 1 in. The disks were taken from the widest part of the leaf, midway between the mid-rib and the edge, and alternately from the right and left half of successive leaves. The morning samples were taken from the opposite halves of the leaves sampled in the evening. This method of sampling was adopted so as to provide material of approximately the same age for all treatments; leaves younger than the 15th could not be sampled because some of them were too small to allow a complete disk to be punched from one-half of the leaf, and on some plants leaves older than the 21st were necrotic or dead. The mean leaf number per plant at the time of sampling was thirty-two. Disks from duplicate plots were bulked, so that the morning and evening sample for each of the six treatments consisted of eighty disks, and there were twelve samples in all. The fresh weight of a sample varied between 21 and 27 g. On each occasion smaller disks were punched from the 15th and 21st leaves of one plant per plot, to be used for a qualitative test for starch by staining with iodine.

Experiment 2

The plants used for this experiment were grown in pots in the glasshouse. Potting compost was used, and the plants twice received dressings of complete fertilizer.

There were three groups, treated as randomized blocks: (1) sown on 27 July; (2) sown on 4 August; (3) sown on 11 August. Each group contained eighteen plants, variety Kleinwanzleben E, and half of them were infected with yellows virus by aphis inoculation 10–14 days after germination.

Samples of leaf lamina were taken at 16.30–18.30 hr. on 5 October 1948, and again at 10.00–12.00 hr. on 6 October. During the intervening period the plants were kept in darkness. Two sets of samples were taken, to provide independent measures of translocation from young and old leaves. One set was taken from the oldest unwilted leaf on each plant and the two next youngest leaves (usually the 4th, 5th and 6th leaves in order of production, but occasionally including either the 3rd or 7th leaves). The other set came from the 4th, 5th and 6th youngest leaves on each plant, counting back from the youngest leaf longer than 1 in. The older infected leaves sampled in the first set were showing yellowing symptoms, less intense than those found in the field, but the younger infected leaves in the second set were still green.

At the evening sampling, two disks each 4·1 sq.cm. in area were punched from one-half of each leaf sampled, and in the morning two disks were taken from the opposite half of each leaf. Thus there were in all twenty-four samples, representing three replications of the combinations of the following treatments:

(*a*) Evening (A) *v.* morning (B) samples.

(*b*) Healthy (H) *v.* infected with yellows (Y).

(*c*) Old *v.* young leaves.

Each sample consisted of fifty-four disks (2 disks/leaf × 3 leaves/plant × 9 plants). The fresh weight per sample varied from 5·6 to 6·6 g. for the older leaves, and from 4·4 to 4·8 g. for the younger leaves.

As soon as all the disks of a sample were cut, they were dropped into boiling 95 % alcohol, and the boiling was continued under a reflux condenser for about ½ hr. After storage in the alcohol extract until it was convenient to proceed with the sugar estimations, the insoluble residue was filtered off, dried at 100°, ground and further extracted with alcohol in a Soxhlet extractor for 8 hr. The residue in the Soxhlet thimble was dried, weighed and preserved for starch estimation. An aliquot of the alcohol extract was evaporated, and the alcohol soluble material was dried at 100° and weighed. The sum of the weights of insoluble residue and of soluble material, calculated from the aliquot, gave the dry weight of the sample.

Another aliquot of the alcohol extract was evaporated under reduced pressure at 30–35°, until all alcohol was removed. The residue was taken up in water and filtered. Sucrose and reducing sugars in the unclarified aqueous solution were estimated by the methods developed by van der Plank (1936) for mangold leaves, using a modification of the Harding and Downs copper reagent. Three determinations of the reducing power of the extract were made, namely, (1) before and (2) after inversion with invertase, and (3) after fermentation with baker's yeast to provide an estimate of the non-sugar reducing substances present. The difference between (1)

and (3) gave the content of reducing sugars, which was expressed as invert sugar, and that between (2) and (1) the content of sucrose.

Starch in the alcohol insoluble residue was determined by the method of Hanes (1936). The residue was treated with boiling alcohol containing 1 % HCl to solubilize the starch, washed with alcohol and dried. Starch was then extracted with boiling water, hydrolysed with β-amylase prepared from barley by Hanes's method, and the resulting maltose estimated by copper reduction.

Results

Effect of infection on carbohydrate content

Experiment 1

The appearance of the subsidiary sample of small disks taken from Exp. 1, after decolorizing with alcohol and staining with iodine, is shown in Pl. 5. The evening samples from all plants were deeply stained, indicating the presence of starch, but in the morning sample only the disks from yellows-infected plants showed intense staining; those from healthy or mosaic-infected plants were either colourless or only slightly coloured. There were some exceptions. Two of the disks in the morning sample from yellows-infected leaves were only slightly stained, but one of them came from a leaf which appeared to contain very little starch in the evening. Also, there was one deeply stained disk in the morning sample from mosaic-infected plants (treatment MN). A reasonable interpretation of these results would be that, while healthy and mosaic-infected leaves lost most of their starch during the night, there is no evidence of appreciable change in the starch content of the yellows-infected leaves. This is the type of evidence from which previous workers have concluded that infection with yellows virus prevents translocation of carbohydrate from the leaf.

Table 1. *Composition of leaf samples from Exp.* 1

A = evening sample; B = morning sample.

Treatment		Dry matter per cent of fresh weight		Per cent of dry matter: Starch		Sucrose		Reducing sugars		Total carbohydrate	
		A	B	A	B	A	B	A	B	A	B
No N	H	13·2	10·9	1·6	0·5	2·5	2·2	5·5	5·0	9·5	7·7
	M	12·5	10·8	1·9	0·5	2·9	2·9	4·8	4·7	9·6	8·1
	Y	15·9	13·5	2·9	1·5	5·4	5·3	19·5	20·2	27·8	27·0
N	H	12·6	10·4	1·5	0·4	2·7	2·6	5·2	4·2	9·4	7·3
	M	12·9	10·5	1·7	0·6	2·2	2·1	4·8	4·8	8·6	7·5
	Y	15·5	13·4	2·7	1·2	4·6	4·6	23·4	23·3	30·7	29·2

Table 1 shows the composition of the leaf samples from Exp. 1, expressed as per cent of total dry matter. As the treatments were not replicated, no estimates of error can be given, but the similarity between the effects of infection in the presence

and absence of nitrogenous fertilizer is evidence of the reliability of the results. The effects of nitrogen on carbohydrate content were small compared with those of yellows-infection. Usually nitrogen depressed carbohydrate content, as might be expected; the most obvious exception was that it increased the reducing sugar content of the yellows-infected leaves.

The starch content of the healthy and mosaic-infected leaves lay between 1·5 and 2 % in the evening samples and decreased to about 0·5 % in the morning samples. The starch content of the yellows-infected leaves was about twice that of the others; it was nearly 3 % in the evening and between 1 and 1·5 % in the morning. Comparison with Pl. 5 indicates that the Sachs test is sensitive to changes of starch content only in the range below about 1·5 % of dry matter. Leaves containing 1·5 % starch or more were so intensely stained that variation in the degree of coloration, and hence in the amount of starch present, were not detectable. At the other end of the scale, leaves with about 0·5 % starch gave only slight coloration. Evidently it is possible for large changes in starch content to occur without being detected by the Sachs test, unless the final concentration falls well below 1·5 %.

The sucrose content of the yellows-infected leaves also was about twice that of the others. The differences in sugar content between healthy and mosaic-infected leaves, and between morning and evening samples were small.

The most remarkable effect of infection with yellows virus was the change in reducing sugar content; this was about four times as great in the yellows-infected leaves as in the healthy or mosaic-infected. In the leaves from yellows-infected plants receiving nitrogen, nearly one-quarter of the dry matter was present as reducing sugars. This effect of yellows on reducing sugar content has not previously been reported, presumably because previous workers have relied on the Sachs test for evidence of differences in carbohydrate content. As with sucrose there was little difference in reducing sugar content between evening and morning samples or between healthy and mosaic-infected leaves. The higher sugar content of yellows-infected leaves is easily detectable by taste.

We may conclude from Table 1 that infection of sugar-beet leaves with yellows virus caused a large increase in their total carbohydrate content; both starch and sucrose were increased, but the greatest effect was on reducing sugars; infection with mosaic virus had little effect on carbohydrate content; total carbohydrate content fell in both healthy and infected leaves during a dark period, mainly through a decrease in starch.

The absolute changes in carbohydrate content during the night are shown in Table 2, where the carbohydrates present in the evening and morning samples are expressed as per cent of R.D.M. Averaging the nitrogen treatments, the losses of total carbohydrate from healthy and yellows-infected leaves were almost identical. In the presence of nitrogenous fertilizer the loss from yellows-infected leaves was greater than from healthy leaves, but in the absence of nitrogen the reverse was true. Thus there is no evidence that translocation of carbohydrate from the yellows-

infected leaves was inhibited. However, infection with mosaic virus did apparently cause a small reduction in the loss of total carbohydrate.

TABLE 2. *Carbohydrate content, per cent of residual dry matter, and loss of carbohydrate during the night, Exp.* 1

A = evening sample; B = morning sample.

		Starch			Sucrose			Reducing sugars			Total carbohydrate		
Treatment		A	B	Loss	A	B	Loss	A	B	Loss	A	B	Loss
No N	H	1·7	0·5	1·2	2·7	2·4	0·3	6·1	5·4	0·7	10·5	8·4	2·1
	M	2·1	0·5	1·6	3·2	3·2	0·0	5·3	5·2	0·1	10·6	8·9	1·7
	Y	4·0	2·0	2·0	7·5	7·2	0·3	27·0	27·6	−0·6	38·5	36·8	1·7
N	H	1·6	0·5	1·1	2·9	2·8	0·1	5·8	4·6	1·2	10·4	7·9	2·5
	M	1·8	0·6	1·2	2·4	2·3	0·1	5·2	5·1	0·1	9·4	8·0	1·4
	Y	3·9	1·8	2·1	6·7	6·5	0·2	33·8	33·0	0·8	44·4	41·3	3·1
Mean	H	1·7	0·5	1·2	2·8	2·6	0·2	5·9	5·0	0·9	10·4	8·1	2·3
	M	2·0	0·6	1·4	2·8	2·7	0·1	5·3	5·2	0·1	10·0	8·5	1·5
	Y	3·9	1·9	2·0	7·1	6·9	0·2	30·4	30·3	0·1	41·4	39·0	2·4

The loss of starch from yellows-infected leaves during the night was greater than that from healthy leaves, but this was offset by a smaller change in reducing sugars. Where no nitrogen was applied, reducing sugars in the yellows-infected leaves apparently increased during the night. In the mosaic-infected leaves, as in those infected with yellows, the change in reducing sugars was small compared with that in the healthy leaves. With all treatments, there was little difference in sucrose between the morning and evening samples. Thus in the yellows and mosaic-infected leaves loss of starch accounted for nearly all the carbohydrate translocated, but in the healthy leaves nearly half the total carbohydrate loss was at the expense of reducing sugars.

Experiment 2

The composition of the leaf samples in Exp. 2 is given as per cent of total dry matter in Table 3, and as per cent of R.D.M. in Table 4. The structure of this experiment was such that the main treatment comparisons require three separate estimates of error based on the variance (1) between plants, (2) between leaves on the same plant, and (3) within leaves, appropriate for the comparisons (1) healthy *v.* infected plants, (2) old *v.* young leaves, and (3) evening *v.* morning samples, respectively. It was considered unnecessary to include standard errors for all possible comparisons in the tables, and only those appropriate to the differences between evening and morning samples in Table 4 have been given.

Table 3 shows that infection with yellows virus greatly increased total carbohydrate per cent of dry matter in the old leaves. This was accounted for mainly by a more than threefold increase in reducing sugars, but sucrose per cent of dry matter was also considerably higher in the infected than in the healthy leaves. In contrast

with Exp. 1, infection had little effect on the starch content of the old leaves; starch per cent of dry matter was actually slightly less in the infected than in the healthy.

TABLE 3. *Composition of leaf samples from Exp. 2. (Mean of three replicates)*

A = evening sample; B = morning sample.

Treatment		Dry matter per cent of fresh weight		Per cent of dry matter: Starch		Sucrose		Reducing sugars		Total carbohydrate	
		A	B	A	B	A	B	A	B	A	B
Old leaves	H	12·7	10·4	2·1	0·5	3·3	1·6	3·0	2·3	8·4	4·4
	Y	14·8	12·7	1·7	0·4	6·4	2·5	10·6	12·3	18·7	15·2
Young leaves	H	17·3	15·1	4·3	1·4	5·7	1·8	2·5	3·1	12·5	6·3
	Y	15·3	12·6	4·1	0·6	2·5	1·0	5·4	2·8	12·0	4·4

TABLE 4. *Carbohydrate content, per cent of residual dry matter, and loss of carbohydrate during the night, Exp. 2. (Mean of three replicates)*

A = evening sample; B = morning sample.

Treatment		Starch			Sucrose			Reducing sugars			Total carbohydrate		
		A	B	Loss	A	B	Loss	A	B	Loss	A	B	Loss
Old leaves	H	2·3	0·5	1·8	3·6	1·7	1·9	3·3	2·4	0·9	9·2	4·6	4·6
	Y	2·1	0·5	1·6	7·9	3·0	4·9	13·0	14·5	−1·4	23·0	18·0	5·0
Young leaves	H	5·0	1·5	3·5	6·6	2·0	4·6	2·8	3·3	−0·5	14·4	6·7	7·6
	Y	4·7	0·7	4·0	2·8	1·0	1·8	6·2	3·0	3·2	13·7	4·6	9·0
S.E.				0·29			0·71			0·59			0·61
Mean	H	3·6	1·0	2·7	5·1	1·8	3·2	3·0	2·8	0·2	11·8	5·6	6·1
	Y	3·4	0·6	2·8	5·3	2·0	3·3	9·6	8·7	0·9	18·3	11·3	7·0
S.E.				0·20			0·50			0·41			0·43

The young leaves of infected and healthy plants had almost identical total carbohydrate contents in the evening samples, but in the morning samples the infected leaves had significantly less than the healthy. Starch and sucrose per cent of dry matter were both lower in the infected than in the healthy leaves at both sampling times. In the evening samples the reducing sugar content of the infected leaves was double that of the healthy leaves, but the difference disappeared in the morning samples. Thus the tendency to accumulate reducing sugars, which was so conspicuous in the old leaves, was already beginning to develop in the young leaves.

Table 4 shows that the loss of total carbohydrate per cent of R.D.M. during the night from the old leaves was not reduced by infection. The loss from young infected leaves was apparently greater than from young healthy leaves, but the difference was not statistically significant. These results confirm those of Exp. 1 showing that infection with yellows virus did not inhibit or appreciably reduce translocation of carbohydrate from the leaves. The loss from the old healthy leaves was almost equally at the expense of starch and sucrose, and there was also a small but not

significant decrease in reducing sugars. In the old infected leaves the greatest change was in sucrose content, and the reducing sugar content increased significantly during the night. In the young leaves these effects were reversed, the infected leaves lost less sucrose and more reducing sugars than the healthy, and the reducing sugar content in the young healthy leaves did not differ significantly between evening and morning. The loss of starch was not significantly affected by infection in either old or young leaves. The change in total carbohydrate per cent of R.D.M. during the dark period was much greater in Exp. 2 than in Exp. 1, and the difference is attributable mainly to greater loss of sucrose and, in the young leaves, of starch in Exp. 2.

Effect of infection on water content

Table 1 shows that in Exp. 1 the yellows-infected leaves had a higher dry matter content per cent of fresh weight and hence a lower water content than the healthy leaves. A similar result was found in the old leaves of Exp. 2 (Table 3), but in the young leaves infection with yellows decreased dry matter content. Infection with mosaic (Table 1) had little effect on dry matter per cent of fresh weight.

It is of interest to inquire whether the reduced proportion of dry matter in the fresh weight of yellows-infected leaves (excepting the young leaves of Exp. 2) compared with healthy leaves, can be accounted for by the higher carbohydrate content of the infected leaves. If this were so, water content expressed on a basis independent of carbohydrate content should be unaffected by infection. Table 5 shows the ratios by weight of water : total dry matter and of water : residual dry matter in the two experiments. In Exp. 1, the weight of water associated with unit weight of total dry matter in infected leaves was consistently about four-fifths of that in healthy leaves, but the weights of water per unit weight of residual dry matter were nearly identical. In this experiment, therefore, it appears that the effect of yellows-infection on water content relative to fresh weight or total dry matter was wholly attributable to the higher carbohydrate content of the infected leaves. An alternative way of stating this result is that the fourfold increase in total carbohydrate content caused by yellows-infection did not affect the amount of water present; hydration was apparently independent of the concentration of sugars and starch present in the leaf.

The old leaves of Exp. 2 showed similar results. The water contents of healthy and infected leaves differed much less when expressed on the basis of residual dry matter than on total dry matter, but the ratio of water : R.D.M. was decreased slightly by infection in both evening and morning samples. The most probable interpretation of this result is that the water content of the leaves was not affected by the increase in carbohydrate content caused by infection, but was reduced in the infected leaves as a result of a change in the non-carbohydrate constituents of dry matter.

As the carbohydrate content of the young leaves of Exp. 2 was little affected by yellows-infection, it makes little difference whether water content is referred to total dry matter or R.D.M. (Table 5); on either basis, water content was increased by

yellows-infection. Infection apparently altered the non-carbohydrate fraction of dry matter in such a way as to reduce water content, the reverse effect to that found in the old leaves.

Table 5. *Water content of healthy leaves and of leaves infected with yellows*

(*a*) Ratio of water: total dry matter. (*b*) Ratio of water: residual dry matter.

Experiment 1

		No N		N	
		H	Y	H	Y
Evening samples	(*a*)	6·5	5·3	6·9	5·5
	(*b*)	7·3	7·4	7·6	7·9
Morning samples	(*a*)	8·2	6·4	8·6	6·5
	(*b*)	8·9	8·8	9·3	9·1

Experiment 2

		Old leaves		Young leaves	
		H	Y	H	Y
Evening samples	(*a*)	6·9	5·8	4·8	5·5
	(*b*)	7·6	7·1	5·5	6·3
Morning samples	(*a*)	8·6	6·9	5·6	6·9
	(*b*)	9·0	8·1	6·0	7·2

Discussion

The conclusion of previous workers, from the results of the Sachs test and from the supposed occlusion of the phloem by gummosis, that starch accumulates in sugar-beet leaves infected with yellows virus because translocation of carbohydrates from the leaves is prevented, is clearly erroneous. It is true that infected leaves usually have a higher starch content than comparable healthy leaves; this was shown in Exp. 1 and was confirmed in samples taken earlier from the same field plots on several occasions, though the results of Exp. 2 show that it is not invariably so. But the losses of starch and of total carbohydrates from infected leaves during a dark period were as great as, or greater than, those from healthy leaves.

The present experiments confirmed that infection with yellows virus caused a large increase in the carbohydrate content of the older leaves of sugar beet, and showed that, as healthy and infected leaves lost about equal amounts of carbohydrate per g. residual dry matter during a dark period, the effect of infection on carbohydrate content was as great at the end of the period as at the beginning.

The most interesting result is that by far the greater part of the additional carbohydrate in infected leaves consisted of reducing sugars. Dr A. Kleczkowski has kindly examined extracts from healthy and infected leaves for us by paper chromatography. He found that the reducing sugars in both healthy and infected leaves consisted of glucose and fructose in approximately equal amounts, and no other sugars, except sucrose, were detected. Infection, therefore, does not cause

the production of new forms of sugar or increase forms present only as traces in healthy leaves, but merely increases the concentration of the normal constituents of the leaf.

The mechanism of carbohydrate accumulation in yellows-infected leaves remains obscure. We have shown that it cannot be attributed to reduced translocation, and there are no grounds for ascribing it to an increased rate of carbohydrate production. Growth studies on field crops not yet published have shown that the net assimilation rate of sugar-beet plants is reduced by yellows-infection, and the most probable explanation of this is that the rate of photosynthesis is decreased. It is, however, possible, though very unlikely, that net carbohydrate production (excess of photosynthesis over respiration in the leaf lamina) is greater in infected than healthy leaves, the rate of translocation from the leaf lamina being similar in both; if so, the reduced net assimilation rate of infected plants must be due to increased respiration in other parts of the plant than the leaf lamina. It seems more probable that the increased carbohydrate content of infected leaves is caused by changes in the cells of the leaf, possibly affecting the activity of the enzyme systems controlling the inter-conversion of the different forms of carbohydrate, or their conversion into non-carbohydrates, and that it is not merely a passive consequence of a shift in the balance between carbohydrate production in the leaves and its distribution to and utilization in other parts of the plant. Evidence that such changes in the leaf cells may occur is provided by some sap-transmitted viruses (e.g. tobacco mosaic in tobacco), which cause local accumulation of starch in groups of cells at the point of entry of virus, the so-called 'starch lesions'. Further study of carbohydrate metabolism in yellows-infected sugar-beet leaves in comparison with healthy leaves may throw light on the mechanism that controls the concentration of sugars and starch in plant cells, about which little is known.

The authors wish to acknowledge their indebtedness to Miss B. M. Hamlyn and Mr S. A. W. French for help in carrying out the experiments.

REFERENCES

Clinch, P. E. M. & Loughnane, J. B. (1948). Seed transmission of virus yellows of sugar beet (*Beta vulgaris* L.) and the existence of strains of this virus in Éire. *Sci. Proc. R. Dublin Soc.* **24** (n.s.), 307–18.

Hanes, C. S. (1936). The determination of starch in plant tissue, with particular reference to the apple fruit. *Biochem. J.* **30**, 168–75.

Klinkenberg, C. H. (1945). Anatomisch onderzoek van der Vergelingsziekte van Bieten, vergeleken met enkele andere Bietenziekten. *Meded. Inst. Suikerbiet., Bergen-o-Z.*, **2**, 33–65.

Mason, T. G. & Maskell, E. J. (1928). Studies on the transport of carbohydrates in the cotton plant. I. A study of diurnal variation in the carbohydrates of leaf, bark, and wood, and of the effects of ringing. *Ann. Bot., Lond.*, **42**, 189–253.

Quanjer, H. M. (1934). Enkele kenmerken der 'vergelings'-ziekte van suiker- en voederbieten ter onderscheiding van de 'zwarte houtvatenziekte'. *Tijdschr. PlZiekt.* **40**, 201–14.

ROLAND, G. (1936). Recherches sur la jaunisse de la betterave et quelques observations sur la mosaïque de cette plante. *Publ. Inst. belge Amélior. Better.* **4**, 13–35.

VAN DER PLANK, J. E. (1936). The estimation of sugars in the leaf of the mangold (*Beta vulgaris*). *Biochem. J.* **30**, 457–83.

VAN RIEMSDIJK, J. F. (1935). Physiologisch onderzoek van de vergelingsziekte van voederbieten en de schade door deze ziekte teweeggebracht. *Tijdschr. PlZiekt.* **41**, 317–29.

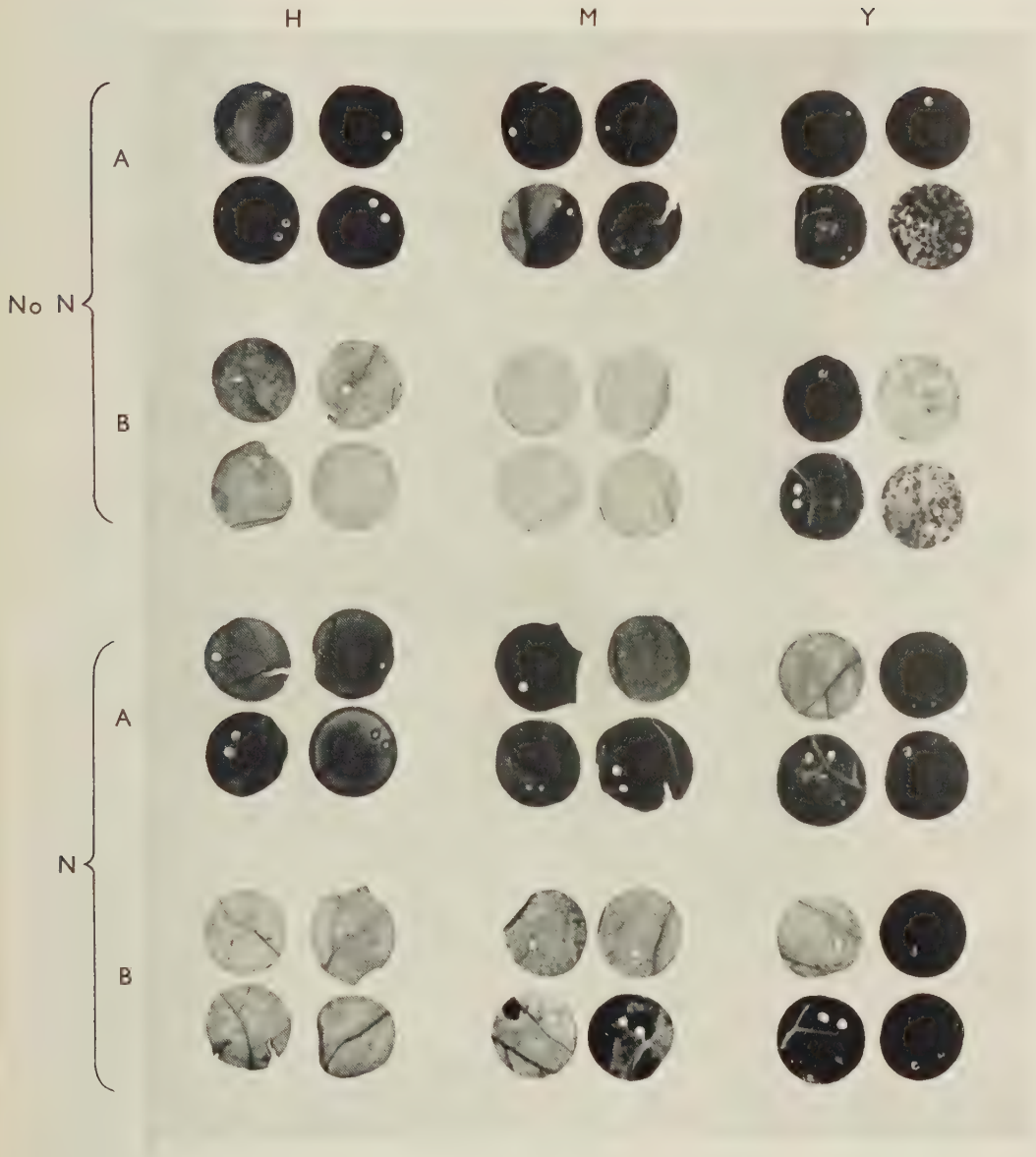
EXPLANATION OF PLATE 5

Disks cut from leaves of (H) healthy sugar-beet plants, (M) plants infected with beet mosaic virus and (Y) plants infected with beet yellows virus, from plots without (no N) and with (N) nitrogenous fertilizer. The disks were decolorized with alcohol and stained with iodine, to show variation in the amount of starch present.

A = disks cut at sunset on 24 September 1946.
B = disks cut soon after sunrise on 25 September 1946.

The left-hand and right-hand pairs in each group of four disks came from different plants. The upper pair were from the 15th leaf and the lower pair from the 21st leaf, counting from the youngest leaf longer than 1 in.

(*Received* 12 *June* 1950)



WATSON and WATSON—*The effect of infection with beet yellows and beet mosaic viruses*

A COMPARISON OF THE GROWTHS OF WILD AND OF CULTIVATED OATS IN MANGANESE-DEFICIENT SOILS

By JOAN M. THURSTON

Rothamsted Experimental Station, Harpenden, Herts

(With Plate 6 and 1 Text-figure)

Two species of wild oats, *Avena fatua* and *A. ludoviciana*, and two varieties of cultivated oats for comparison, were grown to maturity in pots of manganese-deficient soil with and without added manganese.

A. ludoviciana showed different leaf symptoms of manganese deficiency from *A. fatua* and the cultivated varieties. In other respects wild and cultivated oats were similar in their responses to the level of manganese supply, though differences were observed in the severity of the effects. These are summarized in Table 6. The relative susceptibility to manganese deficiency of the wild and cultivated oats differed according to the effect considered. The total dry weight at harvest is an integration of all the preceding effects on growth and is therefore presumably the best single criterion for determining susceptibility to manganese deficiency. On the basis of per cent loss of total dry weight at harvest due to lack of manganese, *A. fatua* is judged less susceptible and *A. ludoviciana* more susceptible than the two cultivated varieties and no distinction can be made between the latter.

The most interesting differences between wild and cultivated oats in response to the level of manganese supply occurred in seed production. Manganese-deficient wild oats showed a smaller reduction in the number of seeds formed but a greater reduction in the size and manganese content of individual seeds than the cultivated varieties.

Manganese deficiency lowered the percentage of viable seeds and the percentage of dormant seeds produced by both *A. fatua* and *A. ludoviciana*.

Introduction

Two species of wild oats occurring at Rothamsted have caused serious infestations of the experimental fields where cereals are grown every year. On Broadbalk *Avena ludoviciana* is prevalent among the winter wheat and *A. fatua* also occurs on some plots, while on Hoosfield the spring barley is infested with *A. fatua*. Information on the factors affecting the growth of these two species of wild oats is therefore of interest, and seeds are readily obtainable for use in experiments. Seeds of both may remain dormant in the soil for several years, making these species difficult to control.

The object of the present work was to compare the susceptibility to manganese deficiency of these species of wild oats with that of varieties of cultivated oats. In cultivated oats a deficient manganese supply induces the condition known as 'grey speck' or 'grey leaf' disease, and causes serious loss of yield (Moore, 1945). Gallagher & Walsh (1943*a*) grew fourteen varieties and species of oats in Irish manganese-

deficient soil and found that *A. fatua* was among the more severely affected forms while *A. strigosa* was almost unaffected. They did not test *A. ludoviciana*.

EXPERIMENTAL METHOD

Ripe seeds of the two species of wild oats were gathered on Rothamsted farm in 1948 and stored in the laboratory until required. Damaged or very small seeds were rejected but no other selection for size, colour, or position in the spikelet was made. The seeds of *Avena fatua* were a mixture of grey, moderately hairy seeds of var. *pilosa* and brown, very hairy seeds of var. *pilosissima*. The yellow-seeded var. *glabrata* is less common at Rothamsted and was absent from this sample.

For comparison with the two species of wild oat two cultivated varieties were selected. One of them, Scotch Potato, has been stated to be very resistant to grey-speck disease (Moore, 1945) and the other, Star, is said to be more susceptible. The seeds of the cultivated oats used had been dressed with a mercurial seed-dressing by the suppliers, but the seeds of the wild species were untreated.

A fen soil (pH 6·8) from Isleham, Cambs, was used. In previous experiments flax and red clover grown in this soil showed severe symptoms of manganese deficiency. From chemical analysis it was estimated that 1·2 g. $MnSO_4$/kg. of soil would be sufficient to prevent the appearance of symptoms and this finding was confirmed by experiments on flax and clover. Accordingly in the present experiment the upper limit of manganese supply was fixed at this level and the effects of one-third and two-thirds of this amount were also tested. The plants were grown in glazed pots each holding 2 kg. of soil.

The treatments therefore consisted of all combinations of:

(*a*) Two wild oat species, *A. fatua* and *A. ludoviciana* and two cultivated varieties, Star and Scotch Potato. For convenience, these will be referred to, collectively, as varieties.

(*b*) Four rates of application of manganese (0, 1, 2 and 3 Mn); 0, 0·8, 1·6 and 2·4 g. $MnSO_4$/pot.

There were two replicate randomized blocks, but the no-manganese treatment was duplicated within each block so that in all there were forty pots.

In addition the four varieties of oats were grown in another manganese-deficient soil, a clay loam (pH 7·3) from Tring, Herts, without the addition of manganese, for comparing symptoms with those shown by plants grown in fen soil.

The manganese sulphate was applied in solution and well mixed with the soil before it was put into the pots, and at the same time a basal dressing of 0·55 g. K_2HPO_4 and 1·00 g. $(NH_4)_2SO_4$/pot was given. The pots were watered with rain-water and the watering was controlled so as to avoid surplus. The pots remained out of doors except in periods of heavy rain or strong wind.

To ensure that only viable and non-dormant seeds were planted, all the seeds, including the cultivated oats, were set to germinate on moist filter-paper in glass germinators on 21 February 1949. The seeds of all the wild species were previously

pricked with a needle to break seed-coat dormancy. As the seeds germinated they were transferred to the pots, those available each day being distributed equally between all pots until each contained ten seedlings. The mean dates of planting the seedlings were: *A. fatua* and Star, 26 February; Scotch Potato, 1 March; *A. ludoviciana*, 5 March.

On 4 April the number of plants per pot was reduced to five. In spite of the seed-dressing, some plants of Star became infected with *Helminthosporium avenae* and had to be removed, so that in two pots of this variety only four plants remained and in another pot only three plants. No further losses of plants occurred between thinning and harvest.

On 12 July the wild oat ears were enclosed in cellophane bags to catch the ripe seeds as they were shed. Ripening continued normally inside the bags and by harvest most of the wild oat seeds had fallen. The cultivated varieties did not shed and were not bagged.

All pots were harvested on 8–10 August by which time most of the ears of all four varieties were ripe.

Results

(1) *Leaf symptoms of manganese deficiency*

Plants of *Avena fatua* and of both varieties of cultivated oats grown with deficient supply of manganese developed the typical symptoms of 'grey speck' disease. The leaves first became chlorotic, and later irregular grey patches appeared. These soon became necrotic and extended until the leaf was dead. The blades of affected leaves frequently bent sharply downwards at about one-third of their length from the stem (Moore, 1945). *A. ludoviciana* showed different symptoms. After the stage of chlorosis, white stripes running between the veins appeared. The stripes later became necrotic, but the lesions were never grey and very rarely irregular in shape. Affected leaves were limp, and never showed the sharp bending found in *A. fatua* and the cultivated varieties.

The first symptoms of chlorosis appeared in most of the plants receiving treatment No Mn about 7 weeks after sowing, but in Scotch Potato they were not observed until a week later. The chlorosis was more severe in the wild than in the cultivated varieties in the period between the 8th and 12th weeks but later it became similar in severity in all four varieties. Star showed grey speck lesions by the 7th week, about a week earlier than *A. fatua* and Scotch Potato; as already noted *A. ludoviciana* never showed this symptom. From the 8th week onwards the lesions became necrotic, and the necrosis was more extensive in the cultivated than in the wild oats. By the 14th week necrosis had proceeded so far in all varieties that many leaves were dead. Thus, the wild oats showed more severe chlorosis, but necrosis developed more rapidly in the cultivated oats.

None of the wild oats receiving manganese developed lesions. With 1 Mn, slight chlorosis developed after 12 weeks. No leaf symptoms appeared where 2 Mn or

3 Mn were supplied. Grey speck lesions developed on both cultivated varieties after 12 weeks where 1 Mn was given, and after 14 weeks where 2 Mn was given, but these treatments suppressed chlorosis. There were no leaf symptoms in the presence of 3 Mn. Thus, a higher rate of manganese application was required to suppress leaf symptoms in cultivated than in the wild oats.

Symptoms developed on the plants grown in Tring soil at about the same time as in those grown in the fen soil. The changes with time in symptom expression and the differences between varieties were also similar in the two soils (Pl. 6).

(2) *Observations made during growth and at harvest*

Measurements of various growth attributes made at intervals usually showed only negligible differences between the three levels of manganese application. In most of the tables, therefore, the data for treatments 1, 2 and 3 Mn have been averaged, so that only means for plants with and without added manganese are given.

Leaf number. For the first 6 weeks no differences in leaf number were found between treatments or species. Later the manganese-treated plants produced leaves more rapidly than the untreated. On 19 April, after 8 weeks' growth (Table 1), the number of leaves per plant was significantly greater on plants with added manganese than on those without, for all varieties. At this time, Star had fewer leaves per plant than the other varieties. Leaf counts were subsequently discontinued.

TABLE 1. *Effect of manganese deficiency on leaf, shoot and ear formation of wild and cultivated oats in fen soil*

Treatment		Mean number per plant					Mean number of days to 50% ear emergence (main crop only)
				Ears			
		Leaves 19 April 8 weeks	Shoots 3 May 10 weeks	9 July 19 weeks	8–10 August At harvest	Increase 9 July–8 August	
Avena fatua	No Mn	7·7	5·7	2·6	4·2	1·6	125·5
	Mn	10·1	8·0	3·1	3·3	0·2	124·0
A. ludoviciana	No Mn	7·8	5·7	1·4	3·4	2·0	126·0
	Mn	10·7	9·8	3·4	3·5	0·1	121·0
Star	No Mn	5·6	4·6	0·8	3·1	2·3	133·0
	Mn	7·2	6·3	2·7	6·1	3·4	111·0
Scotch Potato	No Mn	7·0	5·1	1·0	2·9	1·9	129·5
	Mn	10·0	7·2	2·2	3·5	1·3	119·5
L.S.D. between varieties	No Mn	1·86	1·02	0·77	1·35		
	Mn	1·50	0·84	0·63	1·10		
	Mn–No Mn	2·40	1·32	1·00	1·74		

No Mn: mean of four pots without added manganese.
Mn: mean of six pots, two each of 1 Mn, 2 Mn and 3 Mn.
Ears counted as emerged when at least one spikelet visible either at top or side of sheath. Appearance of awns alone not counted.

Shoot number. Tillering began after about 5 weeks' growth, except in Star which produced no tillers for another 2 weeks. Maximum shoot number was reached in early May. Table 1 gives the mean number of shoots per plant on 3 May, 10 weeks after sowing, when the differences between treatments and species were maximal; similar but smaller differences occurred throughout the period of increasing shoot number. In the presence of added manganese, the wild oats tillered more freely than the cultivated varieties. *A. ludoviciana* produced more shoots than *A. fatua*, and Scotch Potato more than Star. Deficiency of manganese caused a reduction of shoot number in all species; the reduction was nearly equal in *A. fatua*, Star and Scotch Potato, but in *A. ludoviciana* it was almost twice as great as in the other three varieties.

During the tillering phase, *A. ludoviciana* differed conspicuously in its habit of growth from the other varieties. The shoots formed a flat rosette, contrasting with the upright growth of the shoots of the other varieties. This difference was less marked in the No Mn pots, possibly because of the reduced shoot number. When shoot extension began in late May, the difference in growth habit between *A. ludoviciana* and the others was no longer apparent.

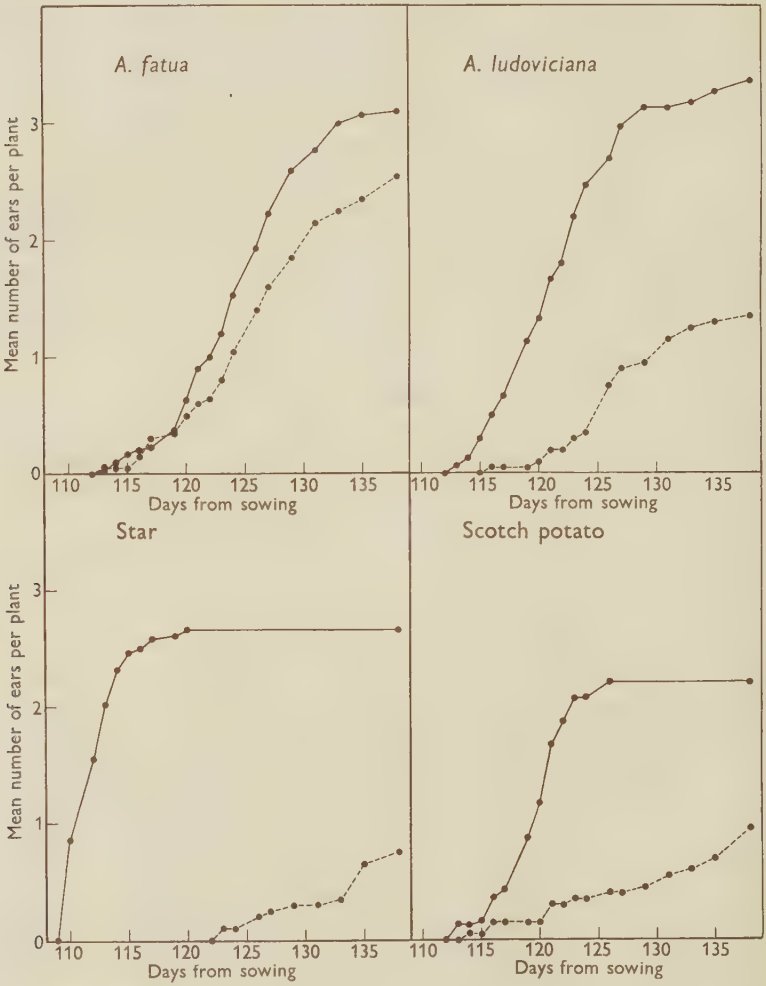
Ear production. In the pots receiving additional manganese, the first ears emerged 16 weeks after sowing. Ears of the cultivated species continued to emerge for 12–14 days, and the number of ears then remained constant for about 3 weeks, but counts made at harvest showed that a number of small ears were produced during the last month of growth, especially in Star (Table 1). Ear emergence in the wild oats continued for 20–25 days, and there was no late ear production in plants receiving additional manganese.

Deficiency of manganese had little effect on rate of ear emergence or of ripe ears per plant in *A. fatua*, but in *A. ludoviciana* the appearance of the first ears was delayed and the subsequent rate of ear emergence was reduced. A few late ears were produced by the manganese-deficient plants of both wild oats. Scotch Potato behaved similarly to *A. ludoviciana*. Manganese deficiency had more severe effects on Star than on Scotch Potato; the beginning of the ear emergence was delayed by 13 days, and the number of ripe ears per plant at harvest was reduced by 70 %, compared with 55 % in Scotch Potato. These differences between varieties in the effect of manganese supply on ear production are shown in Text-fig. 1. The effect of manganese deficiency in delaying ear emergence can conveniently be measured by comparing the dates of 50 % ear emergence of the main crop of ears in plants with and without added manganese (Table 1). The number of days delay in reaching the 50 % stage in the four varieties was as follows:

A. fatua	*A. ludoviciana*	Scotch Potato	Star
1·5	5·0	10·0	22·0

The small later-formed ears produced by the cultivated oats failed to mature and were still green at harvest. The spikelets on many of these little ears were awned, although the large ears produced earlier by the same plants were awnless. This

difference in structure between the large ears that emerged early and reached maturity, and the small late-formed ears that were still green at harvest, was observed both in manganese-deficient plants and in plants receiving added manganese.



Text-fig. 1. The effect of manganese on ear emergence in wild and cultivated oats grown in manganese-deficient soil. – – – – No Mn: mean of four pots. ——— With Mn: mean of six pots.

Culm height. Culm height at harvest was measured from the soil surface to the tip of the terminal spikelet, excluding awns. *A. fatua* was much the tallest variety in all treatments (Table 2 and Pl. 6). With sufficient manganese, *A. ludoviciana* came

second followed by the two cultivated varieties, but where no manganese was added, *A. ludoviciana* and the cultivated oats were equally stunted. *A. ludoviciana* was the only variety in which culm height varied significantly with the amount of manganese added, but even here treatment 2 Mn was sufficient for normal growth.

TABLE 2. *Mean maximum culm height (cm.) at harvest of wild and cultivated oats in fen soil at different levels of added manganese*

Variety	No Mn	1 Mn	2 Mn	3 Mn	Mean of three levels of manganese	Increase due to manganese	% reduction due to manganese deficiency
Avena fatua	98	136(*a*)	134	137	135	37	27·4
A. ludoviciana	72	97	112	114	107	35	36·3*
Star	70	104	101	104	103	33	32·0
Scotch Potato	67	87	90	91	89	22	24·7
Mean	77	106(*b*)	109	112	109	32	
L.S.D.	14·5		(*a*) 9·7 (*b*) 4·8		5·6	15·5	

* Omitting 1 Mn.

All plants grown with manganese produced sturdy culms. Those of the wild oats were more pliable than the shorter and thicker culms of the cultivated varieties, but this seemed to be a varietal characteristic rather than a sign of weakness. Without added manganese the culms of all four varieties were thin and weak. Those which bore a moderate-sized ear bent and broke, but many ears were barren and too light to impose any strain on the culms carrying them (Pl. 6).

Dry weight at harvest. *A. ludoviciana* had a lower total dry weight at harvest than the other varieties at all levels of manganese supply (Table 3). When manganese was applied, *A. fatua*, Star and Scotch Potato did not differ significantly in dry weight,

TABLE 3. *Mean total dry weight, g./pot, of wild and cultivated oats in fen soil at different levels of added manganese*

Variety	No Mn	1 Mn	2 Mn	3 Mn	Mean of three levels of manganese	Increase due to manganese	% reduction due to manganese deficiency
Avena fatua	22·6	47·2(*a*)	50·0	48·0	48·4	25·8	53·3
A. ludoviciana	9·7	34·4	33·2	39·4	35·7	26·0	72·8
Star	16·2	47·2	48·2	49·4	48·3	32·1	66·5
Scotch Potato	16·4	43·9	45·7	48·1	45·9	29·5	64·3
Mean	16·2	43·2(*b*)	44·3	46·2	44·6	28·4	
L.S.D.	4·35		(*a*) 8·37 (*b*) 4·20		4·83	6·51	

Five plants per pot except in three pots of Star where seedlings infected with *Helminthosporium* were removed.

No Mn: mean of four pots.

1 Mn, 2 Mn and 3 Mn: each mean of two pots.

but when it was omitted, the dry weight of *A. fatua* was significantly greater than that of either of the cultivated varieties. Variation of manganese supply over the range from 1 Mn to 3 Mn did not affect dry weight significantly, but there was an indication of a small increase when all four varieties were averaged. The reduction in dry weight caused by lack of manganese did not differ significantly between varieties, but when expressed as per cent of dry weight of plants with added manganese, it was less in *A. fatua* and greater in *A. ludoviciana* than in the cultivated varieties. On the percentage basis, *A. fatua* suffered appreciably less damage from manganese deficiency than the other varieties.

The dry weights of shoots and roots showed similar treatment effects and need not be discussed separately.

Seed production. All varieties benefited both in size and number of seeds from the addition of manganese, though 1 Mn was enough to bring about the full improvement (Pl. 6). *A. fatua* was outstanding for the high number of seeds produced both

TABLE 4. *Seed production of wild and cultivated oats in fen soil at different levels of manganese*

Variety	No Mn	1 Mn	2 Mn	3 Mn	Mean of three levels of manganese	Increase due to manganese	% reduction due to manganese deficiency
			(*a*) Total seeds per pot				
Avena fatua	710	1254(*a*)	1039	1310	1201	491	40·9
A. ludoviciana	212	618	518	694	610	398	65·2
Star	149	908	863	856	875	726	83·0
Scotch Potato	106	506	749	597	617	511	82·8
Mean	294	822(*b*)	795	864	826	532	
L.S.D.	213	(*a*) 288 (*b*) 144			165	270	
			(*b*) Dry weight (g.) of 1000 seeds				
Avena fatua	5·6	9·3(*a*)	12·0	10·3	10·5	4·9	46·9
A. ludoviciana	6·7	14·0	16·2	17·5	15·9	9·2	58·0
Star	6·0	7·7	7·5	8·7	8·0	2·0	24·7
Scotch Potato	7·3	12·8	10·3	12·7	11·9	4·6	38·4
Mean	6·4	10·9(*b*)	11·5	12·3	11·6	5·2	
L.S.D.	2·0	(*a*) 4·7 (*b*) 2·4			2·7	3·4	
			(*c*) Dry weight (g.) of seeds per pot				
Avena fatua	3·8	11·7(*a*)	12·6	13·6	12·6	8·8	69·8
A. ludoviciana	1·5	8·7	8·7	12·1	9·8	8·3	84·7
Star	0·9	7·0	6·6	7·5	7·0	6·1	87·1
Scotch Potato	0·9	6·4	7·4	7·5	7·1	6·2	87·3
Mean	1·8	8·5(*b*)	8·8	10·2	9·1	7·3	
L.S.D.	1·2	(*a*) 4·4 (*b*) 2·2			2·6	2·8	

Five plants per pot except in three pots of Star where seedlings infected with *Helminthosporium* were removed.

No Mn: mean of four pots. 1 Mn, 2 Mn and 3 Mn: each mean of two pots.

with and without added manganese (Table 4). Star with manganese bore significantly more seeds than either *A. ludoviciana* or Scotch Potato. Without manganese these three varieties produced very few seeds compared with *A. fatua*. Star showed the greatest and *A. ludoviciana* the least effect of manganese on the number of seeds formed. The difference between these two responses to manganese was significant but not that between any other pair of varieties.

1000 gr. weights were estimated for the seeds still enclosed in their pales. With adequate manganese the seeds of *A. ludoviciana* were the heaviest and Star the lightest but the difference between Star and *A. fatua* was scarcely significant (Table 4). Without added manganese all varieties were equally poor. The response of *A. ludoviciana* to manganese was significantly greater than that of the other varieties.

The number and weight of seeds produced by normal plants differed widely between varieties. The differences due to manganese were therefore calculated as percentages of the values for normal plants and on this basis it is seen that while manganese deficiency reduced seed number and seed weight in both wild and cultivated oats, the wild oats suffered a proportionately greater loss in seed weight and the cultivated oats in seed number.

The total dry weight of seeds produced depends on the weight of individual seeds and on the number of seeds formed. Table 4 shows that *A. fatua* had the highest dry weight of seeds in all treatments and that with added manganese, *A. ludoviciana* also exceeded the two cultivated oats. Both wild oats showed greater benefit than the cultivated varieties from added manganese.

Seed viability and dormancy. A germination test of seeds from treatments No Mn, 1 Mn and 3 Mn was carried out in March 1950. The germination of Star was negligible, even after dressing with Harvesan. Scotch Potato was little better, giving 20 % germination of No Mn and 25 % of 1 Mn and 3 Mn. These percentages were too low to show any definite effect of manganese on viability. The poor germination of the cultivated varieties may have been due to excessively high temperatures while the seeds were ripening. After the wild oat ears were enclosed in cellophane bags, all the pots were kept in the glasshouse to avoid wind damage. At this time there was continuous hot sunny weather which caused the glasshouse temperature to rise to 80–90° F. The bags may have protected the developing wild oat seeds, as *A. fatua* reached nearly 70 % viability and *A. ludoviciana* probably 60 %.

Both wild oats species were tested with and without pricking, in an attempt to estimate dormancy due to seed-coat impermeability and also total viability. *A. fatua* gave the most reliable results. Both the percentage of viable seeds and the percentage dormancy increased with the amount of manganese supplied to the parent plants:

	No Mn	1 Mn	3 Mn
% viable seeds	28	44	67
% dormant seeds	18	39	64
Dormant as % of viable seeds	64	89	96

The germination of *A. ludoviciana* was unaffected by pricking, and averaged 8 % in No Mn and 18 % in 1 Mn and 3 Mn. After 5 weeks all the pricked seeds of treatment No Mn which had not germinated had decayed and only 6 % of the pricked seeds of treatments 1 Mn and 3 Mn remained sound but ungerminated. The figures for unpricked seeds remaining sound after 5 weeks were 7 % in No Mn, 21 % in 1 Mn and 42 % in 3 Mn. It is possible that the unpricked seeds which neither germinated nor decayed were viable but dormant, and that pricking had caused decay of any seeds which were not ready to germinate immediately, before germination could take place. If this were so, then *A. ludoviciana* like *A. fatua* showed increased viability and dormancy of the seeds from plants grown with additional manganese. Assuming the undecayed seeds to be viable, *A. ludoviciana* gave 15, 39 and 60 % viable seeds in treatments No Mn, 1 Mn and 3 Mn respectively and of these 47, 54 and 70 % were dormant.

Manganese content. The manganese content of shoots and seeds of treatments No Mn, 1 Mn and 3 Mn were determined spectrographically on bulked replicates. Treatment 2 Mn was omitted to save labour, and the roots also were not analysed because it was impossible to be sure that they were free from soil. The manganese content in p.p.m. of dry matter of the shoot (Table 5) varied little with manganese

TABLE 5. *Manganese content at harvest of wild and cultivated oats grown in manganese-deficient fen soil at different levels of added manganese*

	Manganese content (p.p.m. of dry matter)						Total manganese (γ/plant)									Increase in total manganese (γ/plant)	
	Shoots			Seeds			Shoots			Seeds			Shoots + seeds				
Variety	No Mn	1 Mn	3 Mn	No Mn	1 Mn	3 Mn	No Mn	1 Mn	3 Mn	No Mn	1 Mn	3 Mn	No Mn	1 Mn	3 Mn	1 Mn–No Mn	3 Mn–1 Mn
Avena fatua	10	9	12	5	14	22	29	48	63	4	34	60	34	82	123	48	41
A. ludoviciana	11	10	16	5	16	23	14	40	65	2	28	56	16	68	121	52	53
Star	14	8	10	30	25	36	41	52	78	6	35	68	47	87	146	40	59
Scotch Potato	10	10	12	21	23	39	26	56	70	4	29	59	29	85	129	56	44
Mean	11	10	12	15	20	30	28	49	69	4	32	61	32	80	130	48	50

supply. It was slightly higher in *A. ludoviciana* than in the other varieties, but the differences between varieties were not consistent at all levels of manganese supply. The manganese content of the seed, in p.p.m. of dry matter, on the other hand, increased steadily with increase in the amount of manganese applied. The seeds of wild oat species had a lower manganese content than those of the cultivated varieties especially for treatment No Mn.

The weight of manganese in shoots and seeds together increased on the average of all varieties, from about 30γ/plant for treatment No Mn to 130γ/plant for treatment 3 Mn. The greater part of this increase occurred in the seeds, the manganese content of which rose from 4 to 61γ/plant, while that of the shoot rose from 28 to 69γ/plant. Thus, in the absence of added manganese only one-eighth of the manganese taken

up by the plant passed into the seed, compared with nearly one-half in the plants receiving treatment 3 Mn.

A. ludoviciana took up less than the other varieties, both in seeds and shoots, especially when no manganese was given; the difference between *A. ludoviciana* and the other varieties decreased with increasing manganese supply. Star showed the highest manganese uptake. The four varieties did not differ greatly in their ability to absorb manganese from the $MnSO_4$ added to the soil; the increase between treatments No Mn and 3 Mn in total manganese content per plant was slightly greater for *A. ludoviciana* and slightly less for *A. fatua* than for the cultivated varieties.

The most interesting feature of Table 5 is the manganese content of the seeds for treatment No Mn. Evidently, the seeds of the wild species were capable of developing with a lower manganese content than the cultivated, so that although the total amount of manganese available for the seeds was similar in all varieties, it was divided between a larger number of seeds in the wild than in the cultivated varieties and was consequently present in lower concentrations.

TABLE 6. *Summary of the effects of manganese deficiency on different aspects of the growth of wild and cultivated oats*

Criterion	Order of increasing susceptibility
Severity of chlorosis in No Mn	S, P < F, L
Severity of necrosis in No Mn	F, L < S, P
Amount of manganese required to suppress leaf symptoms	F, L < S, P
Reduction in tillering	F, S, P < L
Reduction in culm height	F, S, P < L
Delay in reaching 50 % ear emergence	F < L < P < S
Reduction in number of ripe ears at harvest	F < P < S, L
Reduction in number of seeds	F < L < S, P
Reduction in dry weight of 1000 seeds	S < P < F < L
Reduction in total dry weight of seeds produced	F < L, S, P
Difference in total manganese (γ/plant) between No Mn and 1 Mn	S < F < L < P
Difference in manganese (p.p.m. of dry matter) in seeds between No Mn and 1 Mn	S, P < F, L
Dry weight of whole plant at harvest	F < S, P < L

F = *A. fatua*. L = *A. ludoviciana*.
S = Star. P = Scotch Potato.
The symbol < signifies 'less severely affected than'.

DISCUSSION

The effects of manganese deficiency on different aspects of the growth of wild and cultivated oats are summarized in Table 6. The order of susceptibility to manganese deficiency in which the four varieties are placed depends upon what criterion of susceptibility is used. The total dry weight at harvest is an integration of all the preceding effects on growth and is therefore presumably the best single criterion for determining the susceptibility of oat varieties to manganese deficiency. On the basis of per cent loss of total dry weight at harvest due to lack of manganese, *A. fatua* is judged less susceptible and *A. ludoviciana* more susceptible than the two cultivated

varieties, and no distinction can be made between the latter. If the varieties are graded according to leaf symptoms, the two wild oats resemble one another and contrast with the cultivated varieties. Moreover, the relative susceptibility of wild and cultivated oats varies according to the symptom considered. The wild oats showed less acute necrosis than the cultivated varieties and also required a smaller application of manganese for the complete suppression of leaf symptoms, but on the other hand they showed more marked chlorosis than the cultivated varieties when grown without added manganese. Comparisons of leaf symptoms between *A. ludoviciana* and the other varieties were difficult to make because, unlike the others, *A. ludoviciana* never produced typical grey speck lesions.

Leaf symptoms occurred in both cultivated oats in treatments 1 Mn and 2 Mn but not in 3 Mn, although these varieties showed no difference in growth or dry weight between the three rates of manganese applied. This suggests that the appearance of leaf symptoms may be the most sensitive criterion of the onset of manganese deficiency. However, Scotch Potato in treatment No Mn showed a falling-off in leaf and tiller production before leaf lesions developed. The difference in type of lesions between *A. ludoviciana* and the other varieties also throws doubt on the suitability of leaf symptoms as a criterion for comparing the effect of manganese deficiency in the four oat varieties studied.

The results of this experiment do not agree with Moore's (1945) classification of Scotch Potato as very resistant to 'grey-speck' disease, though Star proved susceptible, as he indicated. Nor do they agree with the conclusions of Gallagher & Walsh (1943*a*, *b*) that *A. fatua* was among the more severely affected varieties of oat and rather more susceptible than Star. The discrepancies between these results may be due partly to the conditions under which the plants were grown, for Gallagher & Walsh (1941) showed that the pH and moisture content of the soil affected the expression of manganese deficiency symptoms in oats. The extent to which leaf symptoms were relied upon in determining susceptibility would also affect the results. The re-selected seed of cultivated oats used in the present experiment may also have differed in susceptibility from the seed of the same variety used by Gallagher & Walsh.

Although in this experiment *A. fatua* appeared slightly more resistant to manganese deficiency and *A. ludoviciana* slightly more susceptible than the two cultivated varieties grown for comparison, the deficiency symptoms differed in intensity rather than in character. In fact the results emphasized the similarity of response to manganese in wild and cultivated oats rather than the difference. The only qualitative difference observed was between the white interveinal leaf-stripes of *A. ludoviciana* and the typical grey speck lesions of *A. fatua* and the cultivated varieties grown in manganese-deficient soil.

The most interesting quantitative differences between the response of wild and cultivated oats to manganese deficiency were associated with seed production. Shortage of manganese reduced both number and size of seeds in all varieties but

the percentage reduction in seed weight was greatest in the two wild oats, while the cultivated varieties showed a bigger percentage reduction in the number of seeds formed. The wild oat seeds in treatment No Mn were very low in manganese content (p.p.m. dry matter) compared with the seeds of plants receiving additional manganese, but the manganese content of seeds of the cultivated varieties was not lower in treatment No Mn than in 1 Mn. (In Star it was actually higher in No Mn.) Thus plants of wild oats lacking manganese produced the highest possible number of seeds, at the expense of the size and manganese content of the individual seeds. Manganese-deficient cultivated oats, on the other hand, produced fewer seeds than plants grown with sufficient manganese, but the seeds of the manganese-deficient plants approached the others in size and manganese content. These differences may be correlated with biological factors affecting the wild and cultivated oats. The ability to produce a large number of seeds in conditions of malnutrition may have survival value for the wild species but is of no agricultural value and may have been lost in the course of selecting the cultivated varieties for the production of large grains.

The application of 1 Mn to the soil increased the total manganese present in the tops (shoots and seeds) from two to four times according to the variety. This increase was associated with a marked increase in dry weight and improvement in growth. The further rise in manganese content between treatments 1 Mn and 3 Mn was not associated with any further improvement in growth or increase in dry weight of *A. fatua* or the cultivated varieties, but it was correlated with an increase in maximum culm height in *A. ludoviciana* and the suppression of leaf symptoms in the cultivated varieties. Both wild and cultivated oats continued to absorb manganese beyond the range of concentrations in which it improved growth, but in this experiment the excess manganese was not toxic. This agrees with the findings of Samuel & Piper (1929). Evidently there is a wide range of manganese concentration in the plant between the limits at which deficiency and toxicity occur. The amount of manganese taken up per unit dose applied to the soil was less at the higher levels than at the lowest, as might be expected and as Steenbjerg & Boken (1938) found in their experiments.

The last point of interest concerns the cultivated oats. In this experiment Star and Scotch Potato occasionally produced both normal and awned ears on the same plant. The awned ears were usually late-formed and always small and developed at all levels of manganese supply. The occurrence of 'fatuoid' ears in crops of cultivated oats is frequently reported and is usually attributed to hybridization with *A. fatua*. Aamodt, Johnson & Manson (1934) studied the progeny of naturally occurring fatuoids and also of artificial crosses between *A. fatua* and cultivated oats which had never produced awned forms. From their experiments and from a review of the literature they concluded that the common fatuoids are probably normal Mendelian segregates from such crosses but that sometimes they may be due to chromosome aberration or gene mutation. However, the behaviour of Star and

Scotch Potato in this experiment introduces the possibility that what appear to be fatuoids in a crop of awnless oats may sometimes be due to inadequate nutrition rather than to genetic causes. Aamodt *et al.* do not mention this possibility though they quote Fischer (1900) who expressed surprise at the spontaneous and quick origin of fatuoids and thought it argued against their origin by natural hybridization.

The assistance of Miss S. G. Heintze and Mr H. H. Le Riche in analysing the soil and plant material and of Dr D. J. Watson in advising on the design of the experiment and the presentation of the data obtained are gratefully acknowledged.

REFERENCES

AAMODT, O. S., JOHNSON, L. P. V. & MANSON, J. M. (1934). Natural and artificial hybridisation of *Avena sativa* with *A. fatua* and its relation to the origin of fatuoids. *Canad. J. Res.* **11**, 701.

FISCHER, M. (1900). Winterhafer. *Fühlings landw. Ztg.* **49**, 718, 766, 806.

GALLAGHER, P. H. & WALSH, T. (1941). Investigations on grey speck disease in oats on some Irish soils. *Proc. R. Irish Acad.* **46**B, 143.

GALLAGHER, P. H. & WALSH, T. (1943*a*). The susceptibility of cereal varieties to manganese deficiency. *J. agric. Sci.* **33**, 197.

GALLAGHER, P. H. & WALSH, T. (1943*b*). The influence of manganese on the growth of cereals. *Proc. R. Irish Acad.* **49**B, 187.

MOORE, W. C. (1945). Cereal diseases. *Bull. Minist. Agric. Lond.* no. **129**, 33.

SAMUEL, G. & PIPER, C. S. (1929). Manganese as an essential element for plant growth. *Ann. appl. Biol.* **16**, 493.

STEENBJERG, F. & BOKEN, E. (1938). Karforsøg til Belysning af nogle Havresorters Gødskning med Mangansulfat. *Tidsskr. Planteavl.* **43**, 819.

EXPLANATION OF PLATE 6

Wild and cultivated oats grown in manganese-deficient soils with and without added manganese, photographed 21 July 1949, at 21 weeks from sowing.

Figs. 1–4. In fen soil. Left to right: No Mn, 1 Mn, 2 Mn, 3 Mn.

Fig. 1. *Avena fatua*. Fig. 2. *A. ludoviciana*. Fig. 3. Star. Fig. 4. Scotch Potato.

Fig. 5. In Tring soil without added manganese. Left to right: *Avena fatua*, *A. ludoviciana*, Star, Scotch Potato.

(*Received* 22 *June* 1950)

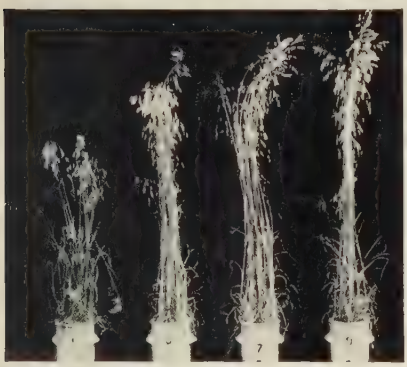


Fig. 1.



Fig. 2.

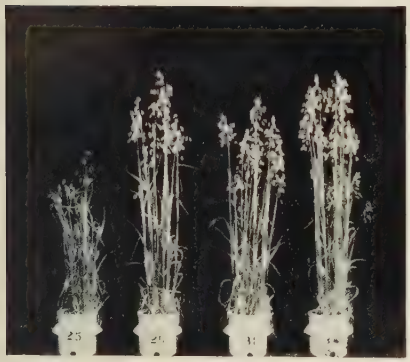


Fig. 3.

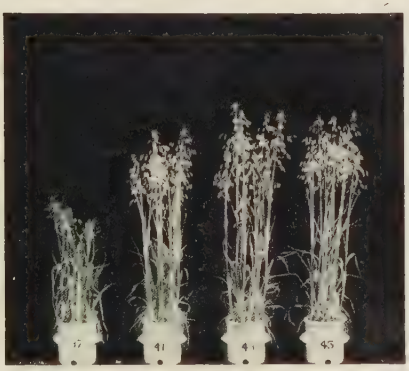


Fig. 4.

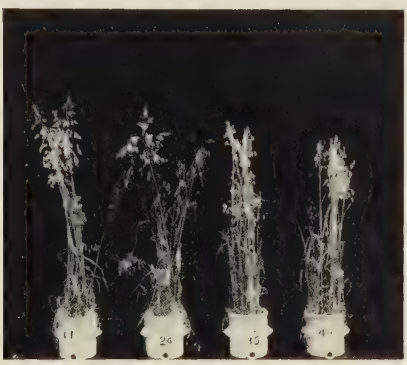


Fig. 5.

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Ordinary Meeting of the Association held on Friday, 21 April 1950, in the Imperial College of Science and Technology, London; the President, Mr G. Fox Wilson, in the Chair.

Symposium on marine biology

The following papers were read and discussed:

1. Forecasting the herring fishery. By Dr W. C. Hodgson.
2. Echo-sounding and pelagic fishes. By Mr I. D. Richardson.
3. The Essex oyster fisheries. By Dr E. W. Knight Jones.
4. Oyster pests. By Mr P. R. Walne.
5. Oyster culture at Conway. By Dr H. A. Cole.

Summer Meeting of the Association held in Scotland, 4–7 July 1950, with Edinburgh as headquarters; the President, Mr G. Fox Wilson, in the Chair during the paper-reading sessions.

Symposia on biological aspects of hill-land farming and on potato and fruit crops in Scotland

The following papers were read and discussed:

1. The relation of bracken control to hill-land farming. By Prof. K. W. Braid.
2. The wintering problem with special reference to cattle. By Dr J. W. Gregor.
3. The tick problem in Scotland. By Dr J. Allan Campbell.
4. Tick-borne diseases of sheep in Scotland. By Dr Angus Foggie.
5. Helminths in Scottish hill sheep. By Dr D. O. Morgan.
6. Recent work on raspberry virus diseases. By Dr C. H. Cadman.
7. Breeding strawberries resistant to disease. By Mr Robert D. Reid.
8. Potato breeding in relation to disease resistance. By Dr William Black.
9. The potato activities of the Department of Agriculture for Scotland, 1918–50. By Dr T. P. McIntosh.

THE RELATION OF BRACKEN CONTROL TO HILL-LAND FARMING

By K. W. Braid, *West of Scotland Agricultural College*

The relatively recent discovery that bracken does not take months to produce new fronds, but merely weeks, and that a tiny plant can grow to a clump of over a yard across in a single year has revolutionized our outlook on the control of bracken. It emphasizes the need of extermination of small colonies and the controlling of the spread of larger areas.

Where ploughing is impossible, cutting still plays the most important part as a method of control. No machine has surpassed the scythe for efficiency, but, with labour costs being what they are, scythe-cutting is prohibitive for large areas. New machines are being designed to cover large expanses more economically and efforts are being made to increase their efficiency as killers of bracken. Even so, pockets of bracken are left along walls, fences and in rough or rocky places. Chemical treatment of such spots is expensive and bares the soil of vegetation, so experiments are being made by means of flushing when this is possible, or burning by vaporized oil or gas. The former method is relatively slow but inexpensive, while the latter has its supply difficulties. Trampling by animals is helpful and manuring to make the grazing attractive has definite results but it is a long-term policy and, as heavy animals avoid rough ground and stones, the method must be intelligently followed up by attacks on the bracken groups they avoid.

The farm of Ballochraggan, Port of Menteith, Perthshire, has been leased from M. T. Buchanan, Esq., of Malling for the past 8 years for research by the Committee appointed to investigate the eradication of bracken. More than 50 % of it was covered in bracken in 1942. In some of the war-years it was difficult to get the bracken cut at the right time, but to-day all the bracken, on the accessible portions, is well under control. Much of the area has been carefully mapped and records of changes in the vegetation are being kept as well as notes of many experiments. Studies are also being made of the plants which are also eaten on such sites (heather, sedges, rushes, and cotton-grass). Nearly twenty Highland and Galloway cows and some 200 ewes are helping in the reclamation. It is hoped to demonstrate some of the experimental work on Friday.

THE WINTERING PROBLEM WITH SPECIAL REFERENCE TO CATTLE

By J. W. Gregor, *Scottish Plant Breeding Station, Edinburgh*

Direct reseeding of rough hill grazings has given some striking increases in *summer* grass yields, but a point which tends to be overlooked is that on the average hill farm the limiting factor to increased stocking is not lack of summer grass but of *winter* keep. The alternative to tackling the hill land proper is to concentrate on raising the output of grass on the low-ground acreage with the primary object of augmenting the supply of winter feed. It was suggested that in order to make the fullest use of such relatively small acreages unorthodox methods of cultivation and utilization might be necessary. Some investigations were described which illustrated the practical possibility of utilizing nutritionally contrasting but complementary types of vegetation, e.g. using protein-rich cultivated grass to supplement a basic diet of inferior quality roughage. The liberal use of nitro-chalk has been a feature of these experiments and grass of very high protein content has been obtained.

THE TICK PROBLEM IN SCOTLAND

By J. Allan Campbell, *Department of Entomology, University of Edinburgh*

The tick problem in Scotland is confined to one species of tick, *Ixodus ricinus* L. With certain notable exceptions, such as the Pentland, Ochil, and Lammermuir Hills, *I. ricinus* is widely distributed throughout the hill areas where it is a conspicuous parasite of sheep and cattle during spring in south-east Scotland, and during spring and autumn in other districts. The tick never becomes established on arable land, and is uncommon on enclosed pastures except where these are adjacent to infested hills. To complete its developmental processes, which take place on the ground, this organism requires an environment with a continuously high relative humidity, and this factor suffices to account for its virtual restriction to hill country. Within that sphere, however, the physical environment plays but a small part in limiting its distribution, since most hill grazings contain sufficiently extensive areas where the microclimate is

suitable. There is no direct correlation between the distribution of herbage associations, and the distribution and abundance of ticks on a particular hill area. Under different circumstances heavy tick populations have been recognized on heather, bracken, rushes, and various types of grassland, and it is apparent that the part played by the vegetation in defining tick distribution operates through its influence on stock movements. Heavily infested zones within a particular grazing are those which are attractive to stock, and where the relative stocking density and consequent host potential are greatest. From the present knowledge of the ecology of the organism, so well adapted as it is to the hill environment with its sheep and cattle stocks, the conclusion is compelled that the practical solution to the problem of infestation of domestic animals can only be achieved through their protection by acaricides. Intensification of work on this aspect is urgently needed, and is proposed in the near future.

TICK-BORNE DISEASES OF SHEEP IN SCOTLAND

By Angus Foggie, *Animal Diseases Research Association, Edinburgh*

Apart from possible loss in condition directly due to tick-infestation, the sheep tick is associated with three diseases in hill sheep.

Of these, louping-ill is a virus disease which produces nervous symptoms followed by death in affected sheep. The virus is carried from one sheep to another by ticks, the nymphal and adult stages being capable of infecting sheep on which they feed. A vaccine has been evolved and is used extensively to protect sheep against the disease. In unvaccinated sheep exposed to tick-bite for the first time losses may exceed 60 % of the flock.

The second condition, tick-borne fever, is caused by a larger parasite of the *Rickettsia* group, which, like louping-ill is carried from sheep to sheep by the tick. On tick-infested pastures, the lambs are infected shortly after birth but the resulting disease is mild. The infection remains in the animal's body throughout life but further attacks of the disease do not occur. If, however, older sheep are exposed to tick-bite for the first time more severe symptoms may occur and in-lamb ewes frequently abort.

The third condition, tick-pyaemia, is a disease of young lambs characterized by abscess formation and lameness. It is caused by a *Staphylococcus*, which is a common wound infector in all animals including man. The germ is not carried by the tick but may gain access to the lamb's body through the tick-bite wound. There is as yet no satisfactory means of preventing or curing this disease.

HELMINTHS IN SCOTTISH HILL SHEEP

By D. O. Morgan, *Department of Zoology, University of Edinburgh*

On pastures carrying a light population of sheep, serious outbreaks of helminthic disease are not likely to occur and this may be the reason why so little attention has been given to the problem of worms in Scottish hill sheep. In Scotland the density may be as low as one sheep to 10 or even 15 acres, but it should be realized, however, that even on these lightly grazed Scottish hills, the concentrated grazing by hill sheep of small areas where the herbage is of fair quality gives a stocking rate per acre which is comparable to that occurring on highly productive low-ground fields.

Extensive studies have been, and are, in progress at the Department of Zoology, of the University of Edinburgh, and the results have shown that there is, in hill sheep, a worm problem of considerable importance. These studies have also shown that there is a clear seasonal pattern in the occurrence of worms in hill sheep. From a low level in January, there is an increase in the worm burden during the period from March to about the end of May or early June; this is followed by a gradual fall. This rise in the worm burden occurs in about 75 % of the sheep and is very clearly defined in fully matured sheep, but shows some

variability in the hoggs. The results have brought out several interesting relationships between parasite and host which have a bearing on the epidemiology of worm disease in the grazing animal. It is also considered that the seasonal pattern outlined above is an essential guide in planning experiments to test programmes of prophylactic dosing.

RECENT WORK ON RASPBERRY VIRUS DISEASES

By C. H. Cadman, *Scottish Raspberry Diseases Investigation, University College, Dundee*

Since 1944, an investigation, sponsored by the Agricultural Research Council, has been in progress into the causes of decline in vigour and crop failure of raspberries in Scotland. Though it is clear that frost, insect pests and fungus diseases all take their toll of the crop, recent work has shown that virus diseases are a major cause of loss.

An unexpectedly large number of distinct virus diseases has been identified as a result of recent work and it has been shown that a number of these are spread in the field by means of aphids, principally the green raspberry aphid, *Amphorophora rubi*.

It has been a marked feature of raspberry growing that a high standard of health and vigour of stocks cannot be maintained solely by roguing out visibly infected plants.

Analysis of virus-induced decline in the variety Lloyd George has now led to a clearer understanding of the situation. Visible mosaic symptoms on the majority of current commercial varieties are the result of infection with any one of six different viruses. The spread of these in field crops can largely be controlled by roguing. Decline in vigour of stocks of Lloyd George may, however, result from combined infection with two other viruses, neither of which produce diagnosable symptoms on infected plants.

The presence of carried viruses in stocks can be detected only by specialized testing methods. The information now available should, however, make possible the production and maintenance of supplies of healthy planting cane.

BREEDING STRAWBERRIES RESISTANT TO DISEASE

By Robert D. Reid, *West of Scotland Agricultural College, Auchincruive, Ayrshire*

Breeding of strawberries has been carried on at Auchincruive for 17 years. The primary object has been to obtain varieties resistant to red core disease, of a type possessing qualities acceptable to commercial growers and propagated under conditions which ensure that they are not seriously infected with other diseases and pests, especially virus diseases and eelworm, when distributed to the trade. Complete resistance to the ordinary strains of *Phytophthora fragariae* has been obtained but new specialized strains able to attack resistant varieties may appear when environmental conditions are specially favourable to the multiplication of these variants.

The combination of all the qualities required in a good commercial variety does not appear often. The recent successful introduction, Auchincruive Climax, was selected from a batch of 14,000. Steps taken to ensure that new introductions will be free from infection by virus diseases include grafting a clone of each promising variety in an early stage of its test to indicator plants (*Fragaria vesca*) raised from seed in sterilized soil. Such clones as are found to be virus-free are propagated in isolation. Strawberry eelworm has interfered with breeding work and detailed observations of the symptoms, recovery versus severe damage or even death have been carried out at Auchincruive. The main problem of the parasitology, etc. is being studied by Dr Franklin at Rothamsted and also at East Malling Research Station.

POTATO BREEDING IN RELATION TO DISEASE RESISTANCE

By William Black, *Scottish Plant Breeding Station, Edinburgh*

Natural resistance to practically all the diseases affecting the potato crop has been found amongst the numerous species and varieties which make up the potato family. This resistance

is a complex, represented by the combined effect of a number of morphological and physiological characters of the plant. Some are concerned with the protection of the plant against infection while others determine the reaction of the plant after infection has taken place. One of the latter, the sensitiveness of the plant cells to the invading organism, is easily recognized and has a direct bearing on the amount of damage suffered. Extremely sensitive cells, i.e. hypersensitive, are killed so quickly after infection that the parasite is enclosed and subsequently dies within the necrotic tissue which it creates. Such hypersensitive plants suffer no appreciable harm and may be regarded as virtually immune from the particular disease under ordinary conditions of growth.

The particular importance of this hypersensitive condition is that genetically it behaves as a dominant character and is inherited in Mendelian fashion. Hypersensitiveness to the wart fungus and to viruses *A* and *X* are already present in commercial varieties and it is a relatively simple matter to breed new types possessing all these characters in combination. In the case of virus *Y* the necrotic response which certain varieties and seedlings exhibit appears to be fully effective only in the presence of a high degree of resistance to infection. None of our commercial varieties provides these conditions but it seems possible by breeding methods to intensify resistance to infection to the desired degree. So far, no species or variety has been found to be hypersensitive to the leaf-roll virus but in certain varieties resistance to infection provides a valuable protection against the disease.

Effective resistance to the blight fungus is not present in ordinary commercial varieties and must be introduced from certain wild Mexican species, the most promising of which is *Solanum demissum*. This species is hypersensitive to the parasite. Although the utilization of wild species involves prolonged breeding operations to obtain seedlings of commercial standard the greatest obstacle to success is the ability of the fungus to mutate and so produce new biotypes of greater virulence than those affecting commercial crops. It has been found in the past, that the production of resistant varieties has been followed by the appearance of new strains of the disease capable of attacking them.

The hypersensitive condition in *S. demissum* is governed by a series of genes. Five of these genes controlling resistance in the foliage have been identified. They are inherited in simple Mendelian fashion and each produces a hypersensitive condition which is effective only against a particular group of biotypes of the disease. In the course of breeding, these genes are unavoidably dispersed but may be reassembled by subsequent hybridization. The strains so far examined have been found to fall into two qualitatively different groups within which only quantitative differences have been observed. The combination of the two genes which confer resistance to the most virulent strain in each group has been found to provide complete protection against all strains at present known.

In a comprehensive breeding programme, it is necessary, not only to bring the various resistance qualities together, but to include also the numerous commercial characters which constitute an economic variety.

THE POTATO ACTIVITIES OF THE DEPARTMENT OF AGRICULTURE FOR SCOTLAND, 1918–50

By T. P. McIntosh, *Scottish Plant Breeding Station, Edinburgh*

In outlining the main activities of the Department, the speaker showed how these have been natural consequences of the introduction of the Registration and Inspection Schemes at the termination of the first World War.

The problems arising from the two schemes have been numerous and varied. The speaker dealt in detail with some of the important items such as varieties, variations and diseases and described the progress made.

In conclusion, he appealed for the closest possible co-operation between scientists engaged on seed production and those working on ware problems, indicating how, in his opinion, this could best be ensured.

REVIEWS

Reports of the Forest Products Research Board; with the Reports of the Director of Forest Products Research for the years 1939–1947. H.M.S.O. 1950. 3*s.* 6*d.*

This report covers the work of the Forest Products Research Laboratory at Princes Risborough for the war years. It was clear from the first that the war posed many problems which only this Laboratory could solve. There was first the severe constriction of supplies and the necessity of using home-grown timber as far as possible to replace imports; secondly, timber had to be put to many special war uses, about which our knowledge was inadequate.

But the duty of the Laboratory did not end with research; 'the biggest call on the accumulated knowledge of the Laboratory, and the most important service rendered by the officers' was possibly the advisory work which the Laboratory organized, particularly in enabling sawmillers to season their timber more rapidly by means of kilns. Sir Edward Salisbury, the Chairman of the Board, suggests that the more effective dissemination of the results of the Laboratory's work and its contacts with industry are likely to become a more important part of its activities, though this 'should not be brought about at the expense of the provision for research which is needed to ensure a steady flow of new information'.

It is impossible in a short review even to summarize the problems which form the subjects of research. Among many items of information provided it is interesting to learn that when it was found that serious corrosion of armaments and other metal contents resulted from packing in boxes made from unseasoned timber, and that rain-water running off shingle roofs of Western Red Cedar corroded zinc and lead gutters and valleys, a search among sixty wood species revealed only two which rendered water alkaline.

There is evidence that the centre of interest in timber research is moving away from biology and in the direction of physics and chemistry. Great advances have been made in methods of seasoning timber, in laminated wood of many kinds and in the chemical utilization of wood substance. Owing to the great cost of factories which utilize timber in these ways a very extensive supply of timber is required for economic handling. Our meagre timber resources preclude major developments of this kind in Britain; but the Laboratory's researches are likely to be of great value in the Commonwealth, and close collaboration must be maintained in these matters, especially with the Crown Colonies, whose facilities for research fall far behind their industrial needs.

W. E. HILEY

Field Trials II: The Analysis of Covariance. By JOHN WISHART. Commonwealth Bureau of Plant Breeding and Genetics, Technical Communication No. 15. 1950. Pp. 35. 3*s.* 6*d.*

In 1940, Dr Wishart published *Field Trials: Their Layout and Statistical Analysis*, an introduction to modern experimental design for agriculturalists and others whose mathematical knowledge was insufficient to permit their learning from the original statistical papers. This new publication has been prepared to supplement its predecessor, and may be the first of a series treating of special aspects of the subject of field experimentation. Interesting though it is, many readers will wish that the author had not handicapped his great powers of explaining complicated techniques by submitting to the constraint of a short and highly specialized bulletin. The resulting compound of excellent computing instructions, good elementary outlines of the theory on which they are based, and inadequate emphasis on the practical applications and limitations of the methods, may be dangerous in the hands of some of its readers.

This technical communication contains a clear explanation of the meaning of regression and of covariance analysis, with one or more independent variates. A new technique for dealing with correlated samples is proposed, but without full discussion of its interpretation; its dependence upon the scales of measurement of the variates needs to be emphasized as a restriction on its usefulness. The brevity of the monograph prevents that full discussion of the numerical examples without which statistical teaching is incomplete, and the expression of computing instructions in words rather than equations makes for obscurity. Statisticians will prefer to learn the methods of covariance analysis with more attention to theory, agriculturists and biologists are likely to find this publication too difficult. For those who have the patience to read carefully, much can be found here that may not be readily available elsewhere in non-mathematical form: one reader, at least, wishes that the author had repeated his earlier success by writing with the needs and knowledge of biological research workers to the fore.

D. J. FINNEY

Iodine and Plant Life: Annotated Bibliography 1813–1949. Pp. xii + 114. London: Chilean Iodine Educational Bureau. 1950. N.P.

This is the first of a series of annotated bibliographies of the role of iodine in biology and medicine and is concerned with fundamental and applied knowledge of iodine and vegetable life, the latter term being used in its widest sense.

No fewer than 794 separate papers are listed together with a very brief summary of the conclusions covering the period from the discovery of iodine in seaweed ash by Bernard Courtois in 1811 up to the end of 1949.

A general review giving an historical account and summarizing the present state of knowledge concerning such topics as iodine content of marine and land plants, effects of iodine on growth and development of higher plants, bacteria and fungi, and the use of iodine in plant protection, serves to introduce the bibliography which is classified under these general headings.

The appreciable amount of iodine contained in Chilean nitrate of soda focused interest on the effect of iodine on plant growth. Conflicting results have been obtained and no one has been able to demonstrate a disease of plants specifically attributable to shortage of iodine. It seems possible, however, that a culture solution with an extremely low concentration of iodine has not yet been obtained and the question of whether iodine is an essential element for plant growth remains open. There is no doubt that iodides are toxic to plant growth even at a concentration of 1 p.p.m. in the culture solution.

This book provides a useful source of reference for all aspects of the effects of iodine on plant life.

E. C. HUMPHRIES

Physiological Mechanisms in Animal Behaviour

SYMPOSIA OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY NUMBER IV

Edited by
J. F. DANIELLI
and R. BROWN

35*s. net*

CAMBRIDGE UNIVERSITY PRESS

BOOKS ON NATURAL HISTORY AND BIOLOGY

New, Second-hand & Rare

Catalogue 72 (2300 items) on request

WHELDON & WESLEY, LTD.
83/84 BERWICK STREET
LONDON, W.1
Phone GER 6459

Established 1843

THE ANNALS OF
APPLIED BIOLOGY

MICROFILM COPIES OF
OUT-OF-PRINT BACK NUMBERS

Microfilm copies of Vol. 26, Part 1 at 25*s.*; Vol. 27, Part 1 at 20*s.*; and Vol. 28, Part 1 at 15*s.*; also of Vols. 30, 31, and 32 at 50*s.* per volume or 15*s.* a single part including postage.

Other parts may be made available in this form if the demand is sufficient to justify production.

Please address all enquiries to

CAMBRIDGE UNIVERSITY PRESS
BENTLEY HOUSE, 200 EUSTON ROAD, LONDON, N.W.1

THE ANNALS OF APPLIED BIOLOGY

The Annals of Applied Biology is conducted by the Association of Applied Biologists and is published by the Cambridge University Press. The Association exists to further the study of all aspects of Biology and to correlate pure science with practice. Meetings are held about six times a year for informal discussion and the reading of papers. Papers from non-members must be communicated by a Member.

The annual subscription to the Association, which includes a copy of the *Annals*, is 25*s*. (entrance fee 12*s*. 6*d*.), becomes due on 1 Jan. of each year and should be paid to the Treasurer.

The price of the *Annals* to non-members of the Association is £2. 10*s*. 0*d*. per volume. Such subscriptions (payable in advance) and all communications respecting the publication should be sent to The Cambridge University Press, 200 Euston Road, London, N.W. 1.

Enquiries from the U.S.A. should be addressed to the American Branch of the Press at 51 Madison Avenue, New York, 10.

Claims for missing numbers should be made within the month following that of regular publication.

Quotations can be given for binding cases and for binding subscribers' sets; (suspended) also for bound copies of back volumes.

Vols. I to V of the *Annals* will be supplied at the following reduced rates:—To Members of the Association, single volumes 10*s*., single parts 5*s*.; double numbers, 8*s*. 6*d*. Complete sets I–V £1. 10*s*. 0*d*. To non-members of the Association, single volumes 15*s*., single parts 7*s*. 6*d*.; double numbers 10*s*. Complete sets I–V £2. 5*s*. 0*d*.

Notice to Contributors

MANUSCRIPTS FOR PUBLICATION.

Contributors should address all material to Mr R. W. Marsh, Research Station, Long Ashton, Bristol. Manuscripts submitted for publication must be type-written, double spaced with ample margins, on one side only of white opaque paper approximately 13 × 8 in. It is understood that they are not offered to any other Journal for prior or simultaneous publication. Preference will be given to Members' papers: papers from non-members must be communicated by a Member for consideration by the Editors.

Owing to the demand for space and the high cost of printing, authors must drastically condense their manuscripts, in the general interest of all contributors. Numerical results should be presented either as tables or as diagrams, but not both: only such tables and illustrations as are essential can be published. Papers must start with a summary and conform to the usages of the *Annals* in all typographical matters. Contributors will be responsible for any excess over the usual charges allowed for corrections. Papers over 8000 words can be published only in exceptional circumstances. Quantitative biological data too extensive for complete publication should be deposited with the Librarian, British Museum (Natural History), Cromwell Road, London, S.W. 7.

REFERENCES.

References must be listed at the end of the article according to the "Harvard System" as follows: name and initial(s) of author; year of publication in brackets, further distinguished by the addition of small letters *a*, *b*, *c* to the date where more than one paper published by the same author(s) in the same year is cited; exact title of paper; contracted title of periodical as given in *World List of Scientific Periodicals*; volume number in arabic figures; beginning page number of article. In the text references should be denoted by giving the name of the author(s) with the date of publication in brackets, e.g. (Brown, 1937), (Brown, 1937*a*; Jones & Smith, 1942*a*, *b*). In the References *all* names should be given in full but, where more than *two* collaborating authors are quoted in the text, the names are printed in full only at the first citation; after that the first name is followed by *et al.*

ILLUSTRATIONS.

Text illustrations accompanying the papers should be drawn on smooth white Bristol board in Indian ink about twice the size of the finished block. Shading must be indicated by lines or dots. Graphs should be drawn boldly in Indian ink on white Bristol board or on blue-lined white graph paper. All lettering of illustrations or graphs should be inserted clearly in pencil.

SEPARATES.

Fifty separates of each paper are provided free.

Printed in Great Britain at the University Press, Cambridge (Brooke Crutchley, University Printer)